Review Article

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Review on analytical methods for quantification of ADHD drugs in human biological samples

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Abstract: Attention deficit hyperactivity disorder (ADHD) is a common neuro-developmental disorder. The symptoms of ADHD include difficulty in attention, memory and impulse control. Many pharmaceutical formulations (stimulants and non-stimulants) are available on the market to treat ADHD symptoms. The most commonly used drugs for treatment are amphetamine, methylphenidate, atomoxetine, bupropion, guanfacine and clonidine. In the field of pharmaceuticals, bioanalysis is an important tool used for the quantification of drugs and their metabolites present in biological samples using various analytical methods. Although a number of analytical methods were reported for the quantification of these drugs in biological samples of experimental animals, due to species differences, it is important to develop analytical methods to quantify these drugs in human biological samples to aid forensic and pharmacokinetic studies. In this review, we compile the bio-analytical methods such as spectrophotometry, spectrofluorimetry, mass spectrometry, electrophoresis, liquid chromatography and gas chromatography used for the quantification of ADHD drugs in human biological samples such as blood, plasma, serum, oral fluids, sweat, hair and urine based on earlier published articles from various journals.

Keywords: ADHD, stimulants, non-stimulants, bioanalysis

1 Introduction

Attention deficit hyperactivity disorder (ADHD) is a psychiatric condition and neuro-developmental disorder typically seen in children, with symptoms including lack of

attention, impulsiveness and hyperactivity [1,2]. Worldwide, 8%-12% of children [3] and 2.5% of adults are affected by ADHD [4] and the cause of this disorder is still not clear [5]. Students with this disorder have poor memory and attention, leading to poor academic achievement [6,7] and also affecting their daily activities [8]. Several genetically related studies were conducted in children and adults to explore the genetic risk factors contributing to ADHD [9,10]. Brain imaging studies can assist with ADHD diagnoses, allowing changes in the structure, function and distribution patterns of the brain to be elucidated [11]. Biomarkers can be used for improving the diagnosis and prevention of central nervous system disorders. Some of the important groups of biomarkers were studied for diagnosis of ADHD and its subtypes [12]. Quantitative electro-encephalography (QEEG) measurement of the theta/beta ratio (TBR) is an important tool for ADHD diagnosis with good sensitivity, selectivity and reliability [13,14]. For treatment, approved drugs include both stimulants and non-stimulants at various doses and in various dosage forms. In the class of stimulants, derivatives of amphetamine (AMP) and methylphenidate (MPH) formulations are the most important first line drugs. These drugs have high efficacy. Initially, AMP and MPH increase the central dopaminergic and norepinephrine activity. Due to the specific cellular mechanism of action of AMP and MPH, they can be used to treat depression and anxiety as well as ADHD. In the class of non-stimulants, atomoxetine (ATX) - the selective noradrenaline reuptake inhibitor, bupropion (BUP) - the norepinephrine and dopamine reuptake inhibitor and guanfacine (GNF) and clonidine (CLN) - both selective α_2 -adrenergic receptor agonists are used to treat ADHD [15-24]. Non-pharmacological treatment including counselling, proper diet maintenance, behavioural parenting and classroom-based interventions may also be useful for the management of ADHD [25].

Bioanalysis is described as qualitative and quantitative measurement of drugs and their metabolites present in biological samples such as blood, plasma, serum, saliva, cerebrospinal fluid, breath, sweat, hair and urine. Bioanalysis can elucidate the pharmacokinetics and toxico-kinetics of drugs and can lead to further understanding of the applications of the drugs in clinical,

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forensic and bioequivalence studies [26,27]. The steps involved in bioanalysis are sample collection, sample preparation and sample analysis [28]. From this, sample preparation is considered one of the most important steps as it removes interferences and pre-concentrates the analytes of interest to achieve good accuracy and precision. The most important methods for sample preparation include liquid-liquid extraction (LLE), protein precipitation (PP) and solid phase extraction (SPE) [29]. Commonly, after a sample preparation procedure, high performance liquid chromatography coupled with tandem mass spectroscopy [30] is used for detection and quantification of drugs in biological samples.

In this present study, we mainly focussed on analytical methods for the determination of six pharmaceutically important drugs used to treat ADHD i.e., AMP, MPH, ATX, BUP, GNF, and CLN in human biological samples.

2 Chemistry and physio-chemical properties of ADHD drugs

2.1 AMP

AMP belongs to the 'β-phenylethylamines' class of drugs. Its chemical formula is $C_9H_{13}N$ and molecular weight is 135.21 g/mol [31]. It is a weak basic, synthetic drug, existing in two forms, free base form and salt form i.e., AMP hydrochloride (HCl). AMP in its salt form is highly water soluble whereas AMP in its base form shows less solubility in water. It is a chiral drug existing in two enantiomeric forms, S (+)-AMP and R (-)-AMP, respectively [32]. Vree et al. determined the pKa and partition co-efficient (P) values of some AMP like drugs (ethylamphetamine, methamphetamine, etc.). The pKa value of phenylethylamine was 9.88 and 'P' values were 20.8 (chloroform in water) and 0.277 (heptane in water), respectively [33]. AMP showed UV absorbance at 287 nm using n-hexane as the solvent [34]. The mass spectrum of AMP showed ion peaks at mass to charge (m/z) ratios of 91, 119, and 136 [35].

2.2 MPH

MPH is a solid crystalline drug with a systematic name of methyl-2-phenyl-2-(piperidin-2-yl) acetate. Its molecular formula is $C_{14}H_{19}NO_2$, molecular mass is 233.31 g/mol, boiling point is 136°C and melting point is 74°C. The molecule contains 1 hydrogen (H) bond donor, 3 H bond acceptors and 4 rotatable bonds, and fully complies with

Lipinski's rules. The molecule also has two stereo-centres that generate 4 possible configurational isomers. These isomers are divided into two pairs of enantiomers, erythro (2R, 2'R, and 2S, 2'S) and threo (2R, 2'S, and 2S, 2'R) [36]. MPH is soluble in ethyl acetate, ether, etc., and insoluble in water [37]. MPH shows UV-Visible absorbance at 510 nm (sulphanilic acid as the reagent and water as the solvent) and 610 nm (potassium permanganate as the reagent and water as the solvent) [38]. The mass spectrum of MPH showed ion peaks at m/z 56.1 and 84.2 and a precursor ion at 234.1, respectively [39].

2.3 ATX

The chemical name of ATX is (R)-N-methyl-3-phenyl-3-(o-tolyloxy)-propylamine. The salt form of ATX is ATX. HCl, the molecular formula is $C_{17}H_{21}NO$. HCl and the molecular weight is 291.82 g/mol [40,41]. ATX. HCl is a white solid with a solubility of 27.8 mg/mL in water, a melting point of 167°C and a dissociation constant (pKa) of 10.13. ATX is marketed as the R (-) isomer which is more potent than the S (+) isomer [42]. ATX. HCl shows a UV absorbance at 270 nm using double distilled water as the solvent [43]. The mass spectrum of ATX showed ion peaks at m/z 44 and 256 [44].

2.4 BUP

BUP. HCl is the salt form of BUP. It is a white crystalline, weak basic drug with a chemical name of (±)-2-(tert-butylamino)-3'-chloropropiophenone hydrochloride. Its molecular formula is $C_{13}H_{18}ClNO$. HCl, molecular weight is 239.74 g/mol, boiling point is 334.8°C and melting point is 233°C. It belongs to the 'aminoketones' class of drugs with a pKa of 7.9. BUP has a single chiral centre, giving rise to two enantiomeric forms i.e., (+)-BUP and (-) BUP. The solubility of BUP. HCl at room temperature is 312 mg/mL in water, 193 mg/mL in alcohol and 333 mg/mL in 0.1 N HCl [45,46]. BUP. HCl showed a UV absorbance at 270 nm using 0.1 N HCl as the solvent [47]. The mass spectrum of BUP showed ion peaks at m/z 166, 184, and 240 [48].

2.5 GNF

GNF is a phenylacetyl guanidine with a chemical name of N-amidino-Z(2,6-dichlorophenyl)-acetamide. GNF. HCl is a white or off-white, odourless, crystalline, achiral drug. It has a molecular formula of $C_9H_9Cl_2N_3O$.

HCl, molecular weight of 282.5 g/mol and melting point of 226°C. The solubility of GNF. HCl is 0.163 mg/mL in water, 0.420 mg/mL in 0.1 N HCl, 1.265 mg/mL in acetate buffer (pH 4.5), and 1.302 mg/mL in phosphate buffer (pH 6.8) [49,50]. The mass spectrum of GNF showed ion peaks at m/z 60 and 246 [51].

2.6 CLN

CLN. HCl is an imidazoline derivative with a chemical name of 2- (2,6-dichlorophenylimino) imidazolidine hydrochloride and chemical formula of $C_9H_9Cl_2N_3$. HCl. It is a white crystalline powder with a bitter taste and is soluble in 13 parts of water, absolute ethanol, slightly soluble in chloroform and insoluble in ether. The molecular weight is 230.10 g/mol for CLN and 266.6 g/mol for CLN. HCl. The melting point of CLN is 130°C and 305°C for CLN. HCl. The drug is stable in light, air and at room temperature [52]. CLN. HCl shows UV absorbance at 418 nm (thymol blue as the reagent and water as the solvent) and 448 nm (bromophenol blue as the reagent and distilled water as the solvent) [53]. In the mass spectrum, the ions of CLN. HCl showed peaks at m/z 44 and 230 [54].

3 Bioanalytical methods for determination of ADHD drugs in human biological samples

3.1 Spectrophotometry

Spectrophotometry is a routinely used instrumental technique in scientific research. It is used for the quantitative measurement of drugs present in sample solutions by measuring the intensity of light (electro-magnetic radiation) which is absorbed by the chemical substance [55]. Wallace et al. developed a spectrophotometric method for the determination of AMP in oxalated blood, serum, urine and homogenized tissues. The AMP present in biological samples at different concentrations may weakly absorb ultra-violet (UV) radiation during analysis and it was not possible to analyse the drug directly by using a UV spectrophotometer. Thus, selection of suitable solvents was required for analysis. In the study, AMP in biological samples was extracted by LLE using n-hexane as the extracting solvent and the drug was analysed from 250 nm to 360 nm. AMP showed its maximum absorbance (λ_{max}) at 287 nm and the limit of detection (LOD) was found to be 0.5 μ g/mL [34].

Legua et al. developed a direct extractive-spectrophotometric method for the determination of AMP in urine samples using sodium 1, 2-naphthoquinone 4-sulphonate (NQS) as the reagent. The sample preparation was an important process to obtain good selectivity and sensitivity. Thus, the drug was extracted from urine samples by LLE using hexane and was analysed from 400 nm to 500 nm. AMP showed its λ_{max} at 450 nm. The presence or absence of systematic error was evaluated by using Youden method and standard additions method. It was found that, the Youden method showed presence of a constant bias error and the standard additions method showed absence of proportional bias error [56].

Basavaiah et al. described the charge-transfer complexation method for the quantification of BUP in spiked human urine samples. The BUP base (n-electron donor) was reacted with p-chloranilic acid (PCA) or 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone (DDQ), the π -acceptor and formed coloured products. The coloured products were analysed in the visible region and showed λ_{max} at 520 nm and 585 nm with good sensitivity [57]. There is no reported spectrophotometric method for the determination of MPH, ATX, GNF and CLN in human biological samples.

3.2 Spectrofluorimetry

Spectrofluorimetry is an emission spectroscopy technique. It is used for the quantitative measurement of drugs present in sample solutions by measuring the fluorescence intensity emitted from the excited compound at a certain wavelength [58]. Mauri-Aucejo et al. optimised the conditions for determination of AMP in urine samples by fluorimetry using batch and flow injection methods with on-line extraction. Drugs in the biological sample were extracted by LLE using diethyl ether as the solvent. The excitation and emission wavelengths were found to be 260 nm and 277 nm, respectively. The method showed good recovery and reproducibility [59].

Omar et al. determined some anti-depressant drugs from pharmaceutical dosage form and human serum using spectrofluorimetry. In the study, the drug BUP. HCl was reacted with 5-(dimethylamino) naphthalene-1-sulfonyl chloride (dansyl chloride) in the presence of 0.5 M sodium carbonate and formed a fluorescent compound. The excitation and emission wavelengths of this fluorescent compound were 347 nm and 450 nm, respectively. The proposed method was simple, sensitive and cost effective [60].

Wahbi et al. developed a spectrofluorometric method for the quantification of GNF. HCl in spiked human serum

and urine samples. The analyte in the biological samples was extracted using chloroform as an extraction solvent at basic pH and the concentration was measured by a calibration-curve method. In the study, the analyte in an aqueous potassium hydroxide solution was reacted with benzoin in the presence of β -mercaptoethanol and sodium sulphite to form a highly fluorescent compound. The excitation and emission wavelengths of this fluorescent compound were 325 nm and 435 nm, respectively. The reported method was simple, accurate, selective and sensitive [61]. There is no reported spectrofluorometric method for the determination of MPH, ATX and CLN in human biological samples.

3.3 Mass spectrometry (MS)

MS is used to measure the m/z ratio of charged particles. Different types of ion source are used to ionize chemical compounds for the generation of charged molecules or molecule fragments. Based on their m/z ratios, the ions are analysed by mass analysers. MS has become the instrument of choice in analytical chemistry and forensic science [62]. Among the six ADHD drugs, MS methods are reported for only two drugs i.e., AMP and MPH for the quantification from human biological samples. Habib et al. developed a sensitive MS method for the determination of AMP extracted from human urine samples. The analyte was alkali treated and ionised using a headspace dielectric barrier discharge (DBD) ionization method. The ions were detected by ion trap detector and the m/z of AMP was found to be 136. The proposed method was validated and the LOD was found to be 0.05 ng/mL [63].

Yang et al. developed a simple MS method for the quantification of MPH extracted from human urine samples using MPH-D3 as the internal standard (IS). The analyte in urine samples was extracted by LLE and subjected to analysis. Electrospray ionization (ESI) with a polymer microchip was used as the ion source to ionize the analyte and IS. The ions of the analyte and IS were analysed by triple quadrupole and detected using selected reaction monitoring (SRM) mode. Ions were found at m/z 84.1 and 234.2 for the analyte and 84.1 and 237.2 for the IS, respectively. The proposed method was validated and showed good accuracy and precision with no carryover [64].

Wachs et al. developed and validated a novel MS/MS method for the estimation of MPH in human urine samples using MPH-D3 as the IS. Solid phase sorbent extraction cards with an ESI sprayer was used for sample preparation and analysis was by triple quadrupole. The ions were detected using SRM mode with ions found at

m/z 84 and 234 for the analyte and 84 and 237 for the IS, respectively [65].

3.4 Hyphenated techniques

Hyphenated techniques are the combination of one or more techniques and can be used to detect drugs present in samples with good sensitivity and selectivity. Here, we summarize some reported hyphenated methods such as electrophoresis, liquid chromatography and gas chromatography for the determination of six ADHD drugs extracted from various human biological samples.

3.4.1 Electrophoresis

Electrophoresis is defined as migration of charged ions in an electric field. It is a separation method and the rate of migration is dependent on factors such as net charge, size, shape of the molecule, buffer pH and applied electric current [66]. Capillary electrophoresis (CE) is one important analytical method used for the determination of ADHD drugs in human biological samples using different detectors [67]. Meng et al. developed a sensitive and reproducible micellar CE method for the determination of trace levels of AMP present in human hair. The experiment was carried out using an uncoated fused-silica capillary (60.2 cm $\times$ 75 μ m) with 25 mM sodium dodecyl sulfate in 100 mM phosphate buffer (pH 2.9) and 20% (v/v) of methanol (organic additive) as a running buffer for separation. A negative voltage (-18 kV) was applied for good separation. AMP was detected using a UV detector at wavelength of 200 nm. The proposed method was validated and the LOD was 0.05 μ g/mL [68].

Hudson et al. analysed a number of basic and neutral drugs in whole blood samples using capillary zone electrophoresis (CZE). The relative migration of the drugs AMP, MPH, BUP and CLN was recorded at both pH 2.5 and 9.5. The experiment was carried out using a fused-silica capillary (60 cm $\times$ 50 μ m) with 100 mM/L phosphate as the running buffer. The separation voltage was set to 20 or 25 kV and the temperature was set at 20°C or 25°C. The analytes were detected by UV detector at 200 nm [69].

Hyotylainen et al. compared two methods for the determination of AMP in human serum and urine samples. The experiment was carried out using capillaries (67 cm $\times$ 50 μ m) with 0.1 M tricine (pH 8.5) and 0.05 M glycine and 0.05 M sodium dodecyl sulfate (pH 10.5) as the running buffer. The separation voltage was set to 25 kV for quantification and the temperature was maintained

at 20°C. The analyte was detected by UV detector at 220 nm. In the study, micellar electrokinetic capillary chromatography showed good separation and had greater applications than CZE [70].

Boatto et al. developed a CE method for determination of AMP in human whole blood samples using a photo diode array detector (DAD). The drug was extracted from the biological sample using a PP extraction with acetonitrile. The experiment was carried out using an uncoated fused-silica capillary (50 cm × 50 µm) with 100 mM phosphate buffer (pH 2.5) as the running solution. The separation voltage and the temperature were set to 10 kV and 25°C, respectively. The detection wavelength was set at 200 nm and the AMP peak was obtained at 5.3 min. This method was simple and effective [71].

Hercegova et al. developed a capillary isotachopheresis method for the quantification of CLN and another three drugs in control serum and human urine samples. The drugs were extracted from biological samples by both PP using methanol and SPE and were analysed. The separation was carried out in an analytical column (80 × 0.3 mm) using a cationic electrolytic system (10 mM sodium acetate buffer, pH 4.64 and 10 mM β-alanine). The analytes were analysed by an isotachopheretic analyser with a conductivity detector. The method showed good recovery in the range of 87-99% [72].

Boatto et al. developed a simple and validated CE-MS method for the quantification of derivatives of AMP in human urine samples. The derivatives of AMP were extracted from urine samples by SPE. The experiment was carried out using an uncoated fused-silica capillary (120 cm × 75 µm) with 50 mM ammonium acetate with acetic acid (pH 4.5) as a running buffer and the separation voltage was set at 25 kV. ESI was used as an ion source and the MS detection was carried out from m/z 50 to 500 (full scan) in positive ion mode. The method was validated according to the international guidelines [73].

Nieddu et al. proposed the validated CE-MS method for the quantification of derivatives of AMP in human urine samples. The analyte was extracted from urine by SPE. The experiment was carried out using an uncoated fused-silica capillary (120 cm × 50 µm) with 10 mM sodium phosphate monobasic with phosphoric acid (pH 4.5) as a running buffer and the separation voltage was set at 10 kV. ESI was used as an ion source and the mass spectrum was monitored from m/z 100 to 300 (full scan). Derivatives of AMP were found at m/z 210, 224 and 238 in selected ion monitoring (SIM) mode. The method showed good results in terms of accuracy and precision [74].

Bach et al. quantified MPH in human urine samples by both CE-MS and CE-MS/MS methods. The sample was

prepared by LLE using cyclohexane as the extracting solvent. The experiment was carried out using a fused-silica capillary (65 cm × 50 µm, 190 µm) with 100% aqueous 40 mM ammonium acetate and 30% (v/v) ammonium hydroxide (pH 9.0) as a running buffer. The voltage was applied to the anode (20 kV) and to the cathode (4 kV) for separation. ESI was used as an ion source and the ions were analysed by ion trap analyser. The ions of the analyte were detected using SRM mode at m/z 84 and 234. The proposed method was accurate and precise [75]. There is no reported electrophoretic method for quantification of ATX and GNF in human biological samples.

Allen et al. depicted some important analytical techniques used for the determination of racemic mixtures of MPH. CE was one of the techniques used for chiral separation and detection of the drug in biological matrices [76]. CE was also used to study chiral drugs and their metabolites present in biological samples with sensitive detection and elucidation of their enantiomers and enantioselective actions [77-79]. Lee et al. investigated the enantio-selectivity of (±) *threo*-MPH in human plasma samples by CE. The experiment was carried out using an uncoated fused-silica capillary (40 cm × 50 µm) with 50 mM phosphate buffer (pH 3.0) containing 20 mM HP-β-CD as the chiral selector and 30 mM triethanolamine. The voltage was set at 20 kV for separation and the analytes were determined by UV detector at a wavelength of 200 nm. The method was sensitive and enabled a LOD of 600 pg/mL for both enantiomers [80].

Theurillat et al. proposed a CE method for the determination of enantio-selectivity of ± *threo*-MPH in human oral fluids. The enantiomers of MPH were extracted by LLE and separated using uncoated fused silica capillaries (40 cm × 50 µm) with phosphate and triethanolamine (pH 3.0) as the running buffer and 2-hydroxypropyl-β-CD as the chiral selector. The separation voltage was set to 20 kV for analysis and the capillary cartridge and sample holder temperatures were maintained at 25°C and 20°C, respectively. The enantiomers of MPH were detected using DAD at a wavelength of 200 nm [81].

Castro-Puyana et al. developed a CE method with good resolution (≥ 3) for the determination of enantiomers of BUP extracted from human urine samples. In this method, the separation of the analyte was carried out using an uncoated fused silica capillary (50 cm × 75 µm) with 5 mM sulfated-α-CD as the chiral selector and 25 mM phosphate (pH 3.0) as the running buffer. The enantiomers of BUP (excitation at 266 nm and emission at 453 or 513 nm) were detected by phosphorescence with a pulsed Xe-lamp. The method showed good selectivity and sensitivity [82].

3.4.2 Liquid chromatography (LC)

LC is an analytical chromatographic method used to isolate the individual components of a mixture. It separates molecules in a liquid mobile phase using a solid stationary phase. High Pressure Liquid Chromatography (HPLC) is an advanced type of LC. The samples in a solvent are pumped at high pressure and are passed through a column packed with solid particles. The principle of separation is adsorption. The samples are separated based on their affinities towards the stationary phase and detected using various detectors (including UV-Vis, DAD and fluorescence). Currently, LC using reversed-phase silica columns is preferred for pharmaceutical analysis [83].

Talwar et al. developed an HPLC method for the quantification of AMP class drugs in human urine samples. The drugs were separated using a silica HPLC column (25 cm $\times$ 4 mm, 5 μ m) with a mobile phase of hexane:water saturated with ethyl acetate:chloroform:ethanol in the ratio of 145:35:40:20, v/v/v/v at a flow rate of 1 mL/min and detected at two wavelengths 260 nm (UV) and 450 nm (Visible). The method was validated and the LOD for AMP was found to be 60 μ g/L at 260 nm and 105 μ g/L at 450 nm [84].

Hassan developed and validated a reverse phase HPLC method for the simultaneous determination of CLN and other drugs present in human plasma samples. The drug was extracted from plasma by LLE and was separated in an octadecyle ODS YMC (150 $\times$ 46 mm, 3 μ m) column using acetonitrile:10 mM potassium phosphate buffer at a pH 3.3 as the mobile phase in gradient elution mode at a flow rate of 1 mL/min. The UV-Visible detection was recorded at 220 nm and the LOD was found to be 30 ng/mL [85].

Guo et al. developed a reverse phase HPLC method for the determination of ATX in human serum samples. The drug was extracted from serum by LLE using diethyl ether as the extracting solvent. The separation of the analyte was carried out using an Inertsil ODS-3 column (150 $\times$ 4.6 mm, 5 μ m) with acetonitrile:23 mM phosphate buffer, pH 6.6 (39:61, v/v) as the mobile phase and maprotiline as the IS. The flow rate was set at 1 mL/min and detection was by UV at 210 nm. The method was validated and the LOD was found to be 2.5 ng/mL [86].

Baharfar et al. developed an HPLC method for the determination of trace amounts of CLN in human plasma and urine samples. The extracted analyte was separated using an ODS-3 column 147 (25 $\times$ 4.6 mm, 5 μ m) with acetonitrile:10 mM/L phosphate buffer (pH 4.5) as the mobile phase in the ratio of 80:20, v/v with a flow rate of 1 mL/min and UV detection at 210 nm. The method was

validated and the LOD was found to be less than 7 μ g/mL for urine and 11 μ g/mL for plasma [87].

Campíns Falco et al. developed a normal phase HPLC method for the quantification of AMP in urine samples using NQS as a derivatizing agent. In the study, sample preparation by an SPE procedure was more effective than LLE. The separation was carried out using a LiChrospher Si-60 column (125 $\times$ 4 mm, 5 μ m) with ethanol:chloroform:ethyl acetate:n-hexane (1:22:32:45, v/v) as the mobile phase with a flow rate of 2 mL/min and the detection of AMP was recorded at 280 nm and 450 nm using DAD. Validation parameters including accuracy, recovery and precision were also studied [88].

Gaillard et al. developed an HPLC method for the analysis of drugs and toxicants present in human blood and urine samples. They established the retention time and λ_{max} for 684 compounds. Using these as a reference, the test samples were determined, and the method was useful for toxicology studies. The test drugs were extracted from biological samples by LLE and were separated using a Waters Symmetry C8 column (250 $\times$ 4.6 mm, 5 μ m) with Phosphate buffer: acetonitrile as the mobile phase in gradient elution mode. The flow-rate was set between 1-1.5 mL/min and UV spectra were obtained from 200 to 350 nm using DAD. AMP, GNF and CLN drugs had a λ_{max} at 200.5 nm and the retention times were found to be 3.71, 11.38 and 6.12 min, respectively [89].

Zhu et al. developed a reverse phase HPLC method for the determination of MPH in human plasma samples. MPH was extracted from the plasma using LLE. DIB-Cl with 10 mM sodium carbonate (pH 9.0) was added to the extracted drug for derivatization and was separated using a Phenomenex Luna C18 (2) column (250 $\times$ 4.6 mm, 5 μ m) with acetonitrile:water (73:27, v/v) as the mobile phase with a flow rate of 1 mL/min. The MPH was detected using a fluorescence detector and the excitation wavelength (λ_{ex}) was set at 330 nm and emission wavelength (λ_{em}) was set at 460 nm. The proposed method was validated and found to be sensitive and reliable [90].

Stegmann et al. proposed an HPLC method for the quantification of dexamphetamine, MPH and ATX in human serum and oral fluids. The drugs were extracted from the biological samples by LLE and the extracted samples were derivatized with 4-(4, 5-diphenyl-1H-imidazol-2-yl) benzoyl chloride. The separation of derivatized drugs was carried out using a Phenomenex Gemini-NX C18 column (150 $\times$ 4.6 mm, 3 μ m) with water: acetonitrile (gradient elution mode) as the mobile phase at a flow rate of 0.8 mL/min and analysed using fluorescence detection. The λ_{ex} and λ_{em} were set at 330 nm and 440 nm, respectively. The method was validated and the LODs for

dexamphetamine, MPH and ATX were found to be 1.3, 0.6, 16 ng/mL, respectively, for serum samples and 2.5, 0.6, 5.9 ng/mL, respectively, for oral fluid samples [91].

Ulu *et al.* developed a reverse phase HPLC method for the determination of BUP in human plasma and urine samples. The separation of the drug was carried out using an Inertsil C18 column (150 × 4.6 mm, 5 µm) with methanol:water (75:25 v/v) as the mobile phase with a flow rate of 1.2 mL/min and analysis by fluorescence detection at 458 nm (λ_{ex}) and 533 nm (λ_{em}). The proposed method was validated and the LOD was found to be 0.24 ng/mL [92]. Other HPLC methods for the quantification of AMP, MPH, ATX, and BUP in human biological samples [93-108] and their chromatographic conditions are reported in Table 1.

Kataoka *et al.* developed and validated an LC-MS method for the quantification of AMP and its derivatives extracted from human urine samples. The separation of the analyte was carried out using a supelcosil LC-CN column (3.3 cm × 4.6 mm, 3 µm) with acetonitrile:50 mM ammonium acetate (15:85, v/v) as the mobile phase with a flow rate of 0.4 mL/min. ESI was used as an ion source and the analyte of interest (AMP) was detected using MS in SIM mode at m/z 136 [109].

Apollonio *et al.* developed an ultra-performance LC-MS method for the quantification of AMP extracted from human whole blood samples. The separation of the analyte was carried out using a Waters UPLC BEH C18 column (50 × 2.1 mm) with a mobile phase of aqueous pyrrolidine:methanol (52:48, v/v) at a flow rate of 0.4 mL/min. The analyte was ionized by using a positive ESI method and the ions were detected using SIM mode. The analyte was retained at 1.35 min and the m/z was 136 [110].

Tatsuno *et al.* developed a reverse phase LC-MS method for the determination of AMP in human urine samples. The analyte was extracted from urine using an SPE method and separated using an ODS column (150 × 4.6 mm) with acetonitrile:100 mM ammonium acetate (40:60, v/v) as the mobile phase at a flow rate of 1 mL/min. The analyte was analysed by thermospray ionisation with SIM detection and the m/z was 136 [111].

Bogusz *et al.* developed both LC-MS and LC-DAD/UV methods for the quantification of AMP in human serum and urine samples using AMP-D5 as the IS. The analyte was extracted from serum and urine by LLE and was derivatized using phenylisothiocyanate. The separation was carried out using a Superspher Select B ECO cart column (125 × 3 mm) with 50 mM ammonium formate buffer (pH 3.0):acetonitrile as the mobile phase. The mobile phase ratio was 55:45, v/v with a flow rate of 0.9 mL/min for MS detection and 60:40, v/v with a flow rate of 0.8 mL/min for DAD/UV detection (250 nm). An

atmospheric pressure chemical ionisation (APCI) method was used as an ion source and the ions of the analyte and IS were detected using MS in SIM mode. The m/z was 271 for the analyte and 281 for the IS, respectively. The developed LC-MS method was found to be more specific and selective than the LC-DAD/UV method [112].

Marchei *et al.* developed a reverse phase LC-ESI-MS method for the quantification of MPH extracted from human plasma, oral fluids, sweat, urine [113] and hair samples [114] using SIM detection. The IS was 3, 4-methylenedioxypropylamphetamine (MDPA). The analyte of interest was separated using a Thermo Electron-Hipersil Gold next-generation ultra-pure silica column (150 × 4.6 mm; 5 µm) with acetonitrile:10 mM ammonium acetate (gradient elution mode) as the mobile phase at a flow rate of 1 mL/min. The m/z was 234 for the analyte and 222 for the IS, respectively. The developed method was validated and the LOD was 0.31 ng/mL for plasma samples, 0.15 ng/mL for oral fluid samples, 0.14 ng/mL for sweat samples and 0.30 ng/mL for urine samples.

Shinozuka *et al.* developed an LC-MS method for the quantification of 20 anti-depressants in human plasma samples. The analyte (MPH) was separated at a retention time of 5.1 min using an Inertsil C-8 column (150 × 2.0 mm, 5 µm) with methanol:10 mM ammonium acetate (pH 5.0):acetonitrile (70:20:10, v/v/v) as the mobile phase and a flow rate of 0.10 mL/min. Sonic spray ionisation (SSI) was used as an ion source and the ions were detected in SIM mode. The m/z of MPH was determined to be 234. The developed method was validated and found to be accurate and precise [115].

Wolf *et al.* developed a simple, validated LC-ESI-MS method for the quantification of GNF in human urine samples using protriptyline as the IS. The separation of the analyte was carried out using a YMC Basic S column (150 × 2 mm, 5 µm) with methanol:10 mM ammonium formate (60:40, v/v) as the mobile phase and a flow rate of 0.3 mL/min. The analyte and IS were detected using SIM mode and the m/z was 246, 248, and 250 for the analyte and 264 and 265 for the IS, respectively. The proposed method was reliable and accurate [116].

Danafar *et al.* developed and validated a LC-MS method for the determination of CLN. HCl in human plasma samples. The analyte was extracted from plasma by PP and separated using a C18 column (30 × 2.1 mm, 3.5 µm) with acetonitrile:water with 0.2% (v/v) formic acid (60:40, v/v) as the mobile phase at a flow rate of 0.2 mL/min. The analyte was ionised by ESI and detected using multiple reaction monitoring (MRM) mode. The m/z was 213 and 230. This method was useful for pharmacokinetic studies [117,118].

Table 1: HPLC methods for the determination of ADHD drugs in human biological samples.

Biological Samples	Analyte	Stationary phase	Mobile phase	Flow rate	Detection	LOD	Reference
Human urine	AMP	Hibar analytical column (250 × 4 mm, 5 µm)	0.03 M Phosphate buffer (pH 3.0) with 0.05 M methane sulphonic acid: ACN (48:52, v/v)	0.65 mL/min	UV-Visible at 480 nm	40-60 ng/mL	[93]
Human urine	AMP	Europher-100 C18 column (250 × 4.6 mm, 10 µm)	20 mM phosphate buffer with HCl (pH 4.0): ACN (85:15, v/v)	1 mL/min	UV-Visible at 210 nm	2 µg/mL	[94]
Human urine	MPH	C8 HPLC column (250 × 4.6 mm, 5 µm)	Phosphate buffer (pH 4.6): MeOH: ACN (56:40:6, v/v/v)	0.8 mL/min	UV-Visible at 210 nm	15 ng/mL	[95]
Human urine	AMP	Waters Spherisorb C18 ODS2 column (250 × 4.6 mm, 5 µm)	MeOH: 0.05 M ammonium acetate buffer with 0.1% (v/v) TEA, pH 3.9 (gradient elution mode)	-	UV at 210 nm	14 ng/mL	[96]
Human plasma	MPH	ODS reversed phase column (15 cm × 4.6 mm, 5 µm)	ACN: 0.07% (v/v) TEA with conc. Phosphoric acid, pH 3.4 (35:65, v/v)	1.5 mL/min	UV at 192 nm	2.5 ng/mL	[97]
Human plasma	BUP	Reverse phase column (25 cm × 4.6 mm, 5 µm)	0.05 M monobasic potassium phosphate: ACN with 0.007 M sodium heptane sulfonate and 0.01 M TEA, pH 3 (80:20, v/v)	2.3 mL/min	UV at 214 & 254 nm	5 ng/mL	[98]
Human plasma	BUP	Cartridge u-Bondapak C18 column (10 cm × 8 mm, 10 µm)	ACN: water with perchloric acid, pH 3.15 (40:60, v/v)	1.4 mL/min	UV at 250 nm	-	[99]
Human urine	AMP	Luna C18(2) column (150 × 4.6 mm, 5 µm)	0.025% (v/v) phosphoric acid with TEA buffer, pH 3.4: ACN	1 mL/min	DAD at 210 nm	120 ng/L	[100]
Human urine	AMP	Hypersil ODS RP C18 column (250 × 4 mm, 5 µm)	ACN: water (gradient elution mode)	1 mL/min	DAD at 280 & 450 nm	4 ng/mL	[101]
Human plasma	ATX	Agilent SB-C18 column (150 × 4.6 mm, 5 µm)	ACN: 5 mM heptane sulphonic acid buffer with 1% (v/v) of TEA and GAA, pH 4.8 (40:60, v/v)	1 mL/min	DAD at 272 nm	-	[102]
Human plasma	ATX	Agilent XDB-C8 column (150 × 4.6 mm, 3.5 µm)	MeOH: ACN: 10 mM phosphate buffer, pH 3.0 (35:15:50, v/v/v)	1 mL/min	DAD at 215 nm	16.34 ng/mL	[103]
Human plasma	BUP	Agilent XDB-C8 column (150 × 4.6 mm, 3.5 µm)	MeOH: ACN: 10 mM phosphate buffer (pH 3.0) with 20 mM 1-heptane sulfonic acid sodium salt (40:10:50, v/v/v)	1 mL/min	DAD at 214 & 254 nm	24.8 ng/mL	[104]
Human plasma	BUP	Phenomenex Aqua C18 column (250 × 4.6 mm, 5 µm)	MeOH: 0.05 M phosphate buffer with 85% (v/v) phosphoric acid, pH 5.5 (45:55, v/v)	1 mL/min	DAD at 214 & 254 nm	2 ng/mL	[105]
Human urine	AMP	Vercopak Inertsil 5-ODS-80A column, (250 × 3.2 mm, 5 µm)	ACN: water (70:30, v/v)	0.5 mL/min	Fluorescence (λ _{ex} at 343 nm & λ _{em} at 500 nm)	-	[106]
Human plasma	ATX	Phenomenex Luna C18 (2) column (250 × 4.6 mm, 5 µm)	ACN: water (75:25, v/v)	1 mL/min	Fluorescence (λ _{ex} at 318 nm & λ _{em} at 448 nm)	0.3 ng/mL	[107]
Human plasma & urine	ATX	Inertsil C18 column (150 × 4.6 mm, 5 µm)	MeOH: water (85:15 v/v)	1.2 mL/min	Fluorescence (λ _{ex} at 375 nm & λ _{em} at 537 nm)	-	[108]

HCl – Hydrochloric acid, ACN – Acetonitrile, GAA – Glacial acetic acid, TEA – Triethylamine, MeOH – Methanol

Miyaguchi et al. developed and validated an LC-MS/MS method for the determination of AMP extracted from human hair samples. The separation of the analyte was carried out using a Unison UK-C18 column (150 × 3 mm) with ammonium formate in water (pH 3.5):acetonitrile (gradient elution mode) as the mobile phase at a flow rate of 0.3 mL/min. ESI was used to ionise the analyte and analysis was by both orbitrap and ion trap analyser. The m/z was 91.2, 119.2, and 130 by product ion scan analysis [119].

Concheiro et al. developed and validated an LC-ESI-MS/MS method for the quantification of AMP in human urine samples using AMP-D6 as the IS. The analyte was extracted from urine samples by LLE using diethyl ether as the extracting solvent. The analyte and IS were separated using an Atlantis dC18, Intelligent Speed TM column (20 × 2.1 mm, 3 μ m) with ammonium formate buffer (pH 3.0):acetonitrile (gradient elution mode) as the mobile phase at a flow rate of 0.5 mL/min and was detected using SRM mode with a triple quadrupole mass analyser. The m/z was 90.6, 118.9 and 135.9 for the analyte and 92.7 and 142.2 for the IS, respectively [120].

Vaiano et al. developed and validated an LC-MS/MS method for the determination of AMP extracted from human blood samples. The separation of the analyte was carried out using a Zorbax Eclipse Plus C18 column (50 × 2.1 mm, 1.8 μ m) with 5 mM aqueous formic acid and acetonitrile (gradient elution mode) as the mobile phase with a flow rate of 0.4 mL/min. ESI was used as an ion source and the analyte ions were detected using MRM mode. The m/z of the analyte was found to be 91, 119 and 136 [35]. Marjorie Chêze et al. developed and validated an LC-MS/MS method for the determination of AMP extracted from human hair, urine and blood samples using AMP-D5 as the IS [121]. The LC chromatographic conditions and MS detection information are reported in Table 2.

Chou et al. developed and validated an LC-MS/MS method for the quantification of AMP present in human serum samples. The analyte was extracted from serum by a solid phase micro extraction (SPME) method and separated using a Supelco Discovery C18 column (15 cm × 3 mm, 5 μ m) with acetonitrile: deionized water (gradient elution mode) as the mobile phase with a flow rate of 0.2 mL/min. ESI showed better results than APCI and the parent ion of the analyte was detected using SIM mode. The product ions were detected by MRM with m/z 119 and 136 [122].

The determination of AMP in human plasma and oral fluids by LC-ESI-MS/MS was proposed by Wood et al. The analyte was extracted by methanol precipitation and separated using a Hypersil BDS C18 column (100 × 2.1 mm, 3.5 μ m) with 10 mM ammonium acetate buffer:

acetonitrile (75:25, v/v) as the mobile phase and a flow rate of 0.3 mL/min. AMP-D11 was used as the IS. The analyte and IS were detected in MRM mode and the m/z was 91 and 136 for the analyte and 98 and 147 for the IS, respectively [123]. Further LC-MS/MS methods for quantification of AMP from human hair [124-126], urine [127-131], and serum [132,133] samples are reported in Table 2.

Ramos et al. developed an LC-MS/MS method for the quantification of MPH in human plasma samples. The analyte was extracted from plasma samples by LLE and was separated using an Eclipse XDBC18 column (150 × 4.6 mm, 3.5 μ m) with methanol:water with 0.02% (v/v) ammonium trifluoroacetate (80:20, v/v) as the mobile phase at a flow rate of 1 mL/min. APCI was used as an ion source and the analyte was detected using MRM mode. The method was validated, and the m/z of AMP was 83.9 and 234.5 [134].

de Cássia Mariotti et al. developed and validated an LC-MS/MS method for the determination of MPH in human oral fluids using propranolol as the IS. Separation of the analyte and IS was carried out using a Brownlee C18 column (100 × 4.6 mm, 3 μ m) with 10 mM ammonium formate buffer with 0.1% (v/v) formic acid and acetonitrile with ammonium formate (gradient elution mode) as the mobile phases at a flow rate of 0.4 mL/min. ESI was used as an ion source. The analyte and IS were detected using MRM mode with m/z 56.1, 84.2 and 234.1 for the analyte and 183.1 and 260.2 for the IS, respectively [39]. Other LC-MS/MS methods for quantification of MPH in human plasma [135-137], blood, oral fluids [137], hair [138], and urine samples [139,140] are reported in Table 2.

Choi et al. developed and validated an LC-MS/MS method for the determination of ATX and its metabolites in human plasma samples using metoprolol as the IS. The analyte was extracted from plasma samples by LLE using methyl tert butyl ether as an extracting solvent. The analyte, its metabolites and the IS were separated using a Phenomenex Luna C18 column (100 × 2 mm, 3 μ m) with methanol:10 mM ammonium formate buffer, pH 3.5 (90:10, v/v) as the mobile phase at a flow rate of 0.25 mL/min. ESI was used as an ion source. The analytes and IS were detected using MRM mode and the m/z was 44 and 256 for the analyte and 116 and 268 for the IS, respectively [141,142]. Further LC-MS/MS methods for the quantification of ATX extracted from human plasma [44,143-145], blood, oral fluids, sweat, urine [146], and hair [147] samples are reported in Table 2. Montenarh et al. developed and validated an LC-MS/MS method for the determination of 130 drugs extracted from human whole blood, plasma, serum, hair, liver tissues, gastric contents and urine samples. ATX, BUP and MPH were extracted

Table 2: LC-MS/MS methods for the determination of ADHD drugs in human biological samples.

Analyte	IS	Stationary phase	Mobile phase	Flow rate	Ion Source	Detection mode	m/z	Reference
AMP	AMP- D5	Uptisphere ODB C18 column (150 × 2.1 mm, 5 µm)	2 mM formate buffer (pH 3.0): ACN (80:20, v/v)	250 µL/min	ESI	SRM	65, 91 & 136 (AMP), 66, 92 & 141 (IS)	[121]
AMP	AMP- D5	Atlantis T3 column (150 × 2.1 mm, 3µm)	2 mM ammonium formate (pH 3.0): ACN (90:10, v/v)	200 µL/min	ESI	MRM	119 & 136.1 (AMP), 124.1 & 141 (IS)	[124]
AMP	AMP- D5	Zorbax phenyl column (50 × 2.1 mm, 3 µm)	20 mM formate buffer (pH 3.0): ACN: MeOH (gradient elution mode)	0.25 mL/min	ESI	MRM	119 & 136 (AMP), 123.9 & 141.2 (IS)	[125]
AMP	AMP- D5	Synergi Polar HPLC column (150 × 2 mm, 4 µm)	0.01% (v/v) acetic acid: MeOH (50:50, v/v)	0.2 mL/min	ESI	SRM	91, 119 & 136.3 (AMP), 93, 124.2 & 141.3 (IS)	[126]
AMP	AMP- D5	Luna C18 column (100 × 2 mm, 3 µm)	25 mM FA with 1% (v/v) ACN: 25 mM FA with 90% (v/v) ACN (gradient elution mode)	0.3 mL/min	ESI	SRM	91, 119 & 136.1 (AMP), 93, 124 & 141.2 (IS)	[127]
AMP	AMP- D8	Symmetry shield RP18 column (150 × 2.1 mm, 5 µm)	Water: ACN with TFA (90:10, v/v)	0.2 mL/min	ESI	MRM	91, 119 & 136 (AMP), 96, 127 & 144 (IS)	[128]
AMP	AMP- D8	Symmetry shield RP18 column (50 × 2.1 mm, 3.5 µm)	2% ammonium hydroxide with MeOH (v/v): ACN with 0.05% (v/v) TFA (gradient elution mode)	0.4 mL/min	ESI	MRM	91, 119 & 136 (AMP), 96, 127 & 144 (IS)	[129]
AMP	4-phenylbutylamine	Supelco Astec chirobioticTM V2 column (250 × 2.1 mm, 5 µm)	MeOH with 0.1% GAA (v/v) and 0.02% ammonium hydroxide (v/v)	0.25 mL/min	ESI	MRM	91, 119 & 136 (AMP), 91, 133 & 150 (IS)	[130]
AMP	4-phenylbutylamine	Thermo Aquasil C18 column (100 × 2.1 mm, 5 µm)	10 mM ammonium acetate in water: 10 mM ammonium acetate in MeOH & ACN (1:1, v/v)	270 µL/min	ESI	MRM	91, 119 & 136.1 (AMP), 124 & 141.2 (IS)	[131]
AMP	AMP- D5	Penta-fluoro phenyl propyl silica column (30 × 2.1 mm, 3 µm)	0.1% FA with 1 mM ammonium formate (v/v): ACN with 0.1% (v/v) FA and 1 mM ammonium formate (gradient elution mode)	200 to 700 µL/min	TIS	MRM	65, 91 & 136 (AMP), 93 & 141 (IS)	[132]
AMP	AMP- D5	Synergi Polar RP column (150 × 2 mm, 4 µm)	1 mM ammonium formate with 0.1% (v/v) FA: MeOH with 0.1% (v/v) FA (gradient elution mode)	0.25 mL/min	TIS	MRM	91, 119 & 164 (AMP), 20, 93.1 & 141 (IS)	[133]
MPH	MPH-D9	C18 column	65% (v/v) MeOH: 0.2% (v/v) FA	-	ESI	SRM	84 & 234 (MPH), 93 & 243 (IS)	[135]
MPH	MPH-D9	SeQuant ZIC-HILIC column (50 × 2.1 mm, 5 µm)	100% ACN: 2% (v/v) FA in water (90:10, v/v)	1 mL/min	ESI	MRM	84 & 234.1 (MPH), 93.1 & 243.1 (IS)	[136]

Analyte	IS	Stationary phase	Mobile phase	Flow rate	Ion Source	Detection mode	m/z	Reference
MPH	MPH-D9	Synergi Polar-RP column (50 × 2 mm, 2.5 µm)	10 mM ammonium formate buffer with 0.05% (v/v) FA: MeOH with 0.05% (v/v) FA (gradient elution mode)	0.3 mL/min	ESI	MRM	84, 56, 174 & 234 (MPH), 93 & 243 (IS)	[137]
MPH	MPH-D9	Zorbax Eclipse Plus C18 RRHD column (100 × 2.1 mm, 1.8 µm)	2 mM ammonium formate with 0.2% (v/v) FA in water and ACN (gradient elution mode)	0.3 mL/min	ESI	SRM	56, 84 & 234.1 (MPH), 93 & 243.2 (IS)	[138]
MPH	AMP- D6	Restek Allure PFP Propyl column (50 × 2.1 mm, 5 µm)	0.02% (w/v) formate in water and ACN (gradient elution mode)	0.5 mL/min	ESI	SRM	56.1, 84.1 & 234.1 (MPH), 93.1, 125.1 & 142.2 (IS)	[139]
MPH	AMP-D8	Capcell Pak MG-II C18 column (150 × 2 mm, 5 µm)	0.2% (v/v) FA in water: ACN (gradient elution mode)	400 µL/min	ESI	MRM	56.3, 84.3 & 234.3 (MPH), 127.1 & 144.1 (IS)	[140]
ATX	ATX-D3	C18 column (20 × 4 mm)	MeOH with 0.025% (v/v) TFA: 0.025% ammonium acetate (v/v)	200 µL/min	ESI	MRM	44 & 256 (ATX), 47 & 259 (IS)	[44]
ATX	R/S-[2H7]-ATX	Brownlee Spheri-5 C18 polyfunctional column (100 × 4.6 mm, 5 µm)	Water: 5 mM ammonium acetate with 47.2 mM FA, 4 mM TFA in ACN and water (85:15, v/v)	1 mL/min	APCI	SRM	44 & 256 (ATX), 44 & 263 (IS)	[143]
ATX	Trimipramine-D3 & Topiramate-D12	Kinetex XB-C18 column (50 × 3 mm, 2.6 µm)	2 mM ammonium formate with 0.2% (v/v) FA in water and ACN (gradient elution mode)	0.4 mL/min	TIS	MRM	44.1, 148 & 256.1 (ATX), 103 & 298.3 (IS- 1), 78 & 350 (IS-2)	[144]
ATX	Duloxetine	YMC-Pack TMS column (40 × 4.6 mm, 5-3 µm)	water: 5 mM ammonium acetate with 47.2 mM FA & 4 mM TFA in ACN & water (85:15, v/v)	0.5 mL/min	ESI	MRM	44, 148 & 256 (ATX), 44, 154 & 298 (IS)	[145- 147]
ATX, BUP, MPH	-	Waters SunFire C18 column (150 × 2.1 mm, 3.5 µm)	10 mM aqueous ammonium formate with 0.1% (v/v) FA (pH 3.4): ACN with 0.1% (v/v) FA (gradient elution mode)	500 µL/min	ESI	MRM	90.9, 115.2, 117.2 & 256.1 (ATX), 130.1, 131.1, 184.1, & 240.1 (BUP) & 84.1, 111.2, 217.3 & 234.2 (MPH)	[148, 149]
BUP	BUP-D6	Chromolith SpeedROD RP-18 monolithic column (50 × 4.6 mm)	8 mM ammonium acetate: ACN (55:45, v/v)	5 mL/min	TIS	SRM	184 & 240 (BUP), 184 & 246 (IS)	[150]
BUP	BUP-D9	Supelco Ascentis RP-Amide column (100 × 4.6 mm, 5 µm)	5 mM ammonium formate buffer (pH 3.5): ACN (gradient elution mode)	0.8 mL/min	TIS	MRM	166.3, 184.3 & 240.2 (BUP)	[151]
BUP	BUP-D9	Acquity BEH phenyl column	MeOH: 0.06% (v/v) ammonia in aqueous solution (42:58, v/v)	0.5 mL/min	ESI	MRM	184.1 & 240.3 (BUP), 185.1 & 249.3 (IS)	[152]
BUP	BUP: HCl-D9	Waters Symmetry C18 column (150 × 4.6 mm, 5 µm)	MeOH: 0.04% (v/v) FA aqueous solution (31:69, v/v)	1 mL/min	ESI	MRM	184 & 240 (BUP), 185 & 249 (IS)	[153]

Analyte	IS	Stationary phase	Mobile phase	Flow rate	Ion Source	Detection mode	m/z	Reference
BUP	Carbidopa	Waters Sun Fire C18 column (150 × 3.5 mm, 3 µm)	0.5% (v/v) aqueous FA: MeOH (gradient elution mode)	0.2 mL/min	TIS	MRM	166.2, 184.2 & 240.3 (BUP), 71.3, 181.2 & 227.2 (IS)	[154]
BUP	Venlafaxine	Zorbax Eclipse XDB-C18 column (150 × 4.6 mm, 5 µm)	20 mM ammonium acetate: MeOH (10:90, v/v)	1 mL/min	TIS	MRM	184.1 & 240.3 (BUP), 260.3 & 278.4 (IS)	[155]
BUP	Venlafaxine	Poroshell 120 EC-C18 (100 × 2.1 mm, 2.7 µm)	0.1% (v/v) FA & ACN (gradient elution mode)	0.5 mL/min	ESI	SRM	184 & 240 (BUP), 121 & 278 (IS)	[156]
BUP	Acetaminophen	Lux 3µ Cellulose 3 chiral column (250 × 4.6 mm)	(25:15:60, v/v/v): (60:30:10, v/v/v) of MeOH, ACN & 5 mM ammonium bicarbonate with 0.1% (v/v) ammonium hydroxide (gradient elution mode)	400 µL/min	ESI	MRM	184 & 240.1 (BUP), 109.9 & 152 (IS)	[157]
BUP	BUP-D9	Hypersil Gold C18 column (50 × 2.1 mm, 1.9 µm)	0.1% FA (v/v): ACN with 0.1% FA (v/v)	10 µL/min	ESI	SRM	131.1 & 240.1 (BUP), 131.1 & 249.1 (IS)	[158]
GNF	GNF-13C, 15N3	Luna C18(2) column (30 × 2.1 mm, 3 µm)	0.02% FA in water (v/v): MeOH (gradient elution mode)	0.5 mL/min	TIS	SRM	60.1 & 246.1 (GNF), 64.1 & 250.1 (IS)	[160]
GNF	GNF-13C, 15N3	Fortis UniverSil C18 column (50 × 2.1 mm, 5 µm)	0.1% FA in water (v/v) and ACN (gradient elution mode)	1 mL/min	TIS	SRM	60.1 & 246.1 (GNF), 64.1 & 250.1 (IS)	[161]
CLN	Donepezil	Hypersil BDS C18 column (50 × 2.1 mm, 3 µm)	MeOH: water with 0.1% (v/v) FA (60:40, v/v)	200 µL/min	ESI	SRM	212.9 & 230 (CLN), 90 & 380.2 (IS)	[163]
CLN	Nizatidine	Hypersil Hypurity C18 column (50 × 4.6 mm, 5 µm)	2 mM ammonium acetate: ACN (20:80, v/v)	0.4 mL/min	TIS	MRM	44.1 & 231.9 (CLN), 155.1 & 332.1 (IS)	[164]
CLN	CLN-D4	Waters Acquity UPLC BEH C18 column (100 × 2.1 mm, 1.7 µm)	Water with 5 mM hexa fluoro isopropanol & ammonium hydroxide (pH. 9): MeOH (gradient elution mode)	250 µL/min	ESI	MRM	44 & 230 (CLN), 48.2 & 234.1 (IS)	[165]
CLN	Tizanidine	Inertsil ODS-3 column (50 × 3 mm, 3 µm)	5 mM ammonium formate buffer with FA (pH 2.8): MeOH (10:90, v/v)	0.25 mL/min	TIS	MRM	44 & 230 (CLN), 44 & 254 (IS)	[166]
MPH	rac-MPH-D3	Astec Chirobiotic V2 column (250 × 2.1 mm, 5 µm)	MeOH with 0.025% (v/v) ammonium acetate and 0.025% TFA (v/v)	0.2 mL/min	ESI	MRM	84.1 & 234 (MPH), 84 & 237 (IS)	[172]
BUP	BUP-D9	Chiralpak α1-acid glycoprotein (AGP) column (100 × 2 mm, 5 µm)	20 mM aqueous ammonium formate (pH 5.0): MeOH (gradient elution mode)	0.22 mL/min	ESI	MRM	184.1 & 240.2 (BUP), 185 & 249.2 (IS)	[173]
BUP	BUP-D9	Chiralpak α1-acid glycoprotein (AGP) column (100 × 2 mm, 5 µm)	20 mM formate buffer, (pH 5.7): MeOH (gradient elution mode)	0.22 mL/min	ESI	MRM	184 & 240 (BUP), 184 & 249.2 (IS)	[174]

ACN – Acetonitrile, TFA – Trifluoroacetic acid, GAA – Glacial acetic acid, FA – Formic acid, MeOH – Methanol

from human biological samples by LLE [148,149]. The chromatographic conditions are reported in Table 2.

Denooz *et al.* developed an ultra-performance LC-MS/MS method for the quantification of BUP and its metabolites in human whole blood samples. The analyte was extracted from blood by SPE and BUP-D9 was used as the IS. The analyte and IS were separated at retention times of 3.76 min and 3.75 min, respectively using a Waters Acquity UPLC BEH phenyl column (100 × 2.1 mm, 1.7 μm) with 2 mM ammonium formate buffer (pH 4):acetonitrile as the mobile phase and a flow rate of 0.4 mL/min. ESI was used as an ion source and the analytes were detected using MRM mode. The method was validated, and the m/z was 166, 184 and 240 for the analyte and 185 and 349.2 for the IS, respectively [48]. Other LC-MS/MS methods for the quantification of BUP in human plasma [150-157] and serum samples [158] are reported in Table 2.

Li *et al.* developed and validated a new method (dried plasma spots) for the quantification of GNF using LC-MS/MS. The analyte was extracted from whole blood by SPE and GNF-¹³C, ¹⁵N₃ was used as the IS. The analyte and IS were separated using a Fortis UniverSil C18 column (50 × 2.1 mm, 5 μm) with 0.1% (v/v) formic acid in water:0.1% (v/v) formic acid in acetonitrile (gradient elution mode) as the mobile phase using a flow rate of 1 mL/min. Turbo ion spray (TIS) was used as an ion source. The analyte and IS were detected using SRM mode with a m/z of 60.1 and 246.1 for the analyte and 64.1 and 250.1 for the IS, respectively [159]. The same researcher also developed a dried blood spot technique for the determination of GNF in human blood samples [160,161] using LC-MS/MS and the LC-MS chromatographic conditions are reported in Table 2.

Martin *et al.* developed an LC-MS/MS method for the determination of GNF in human plasma samples using guanabenz as the IS. The analyte was extracted from plasma by LLE using methyl tert-butyl ether (MTBE) as the extracting solvent. Separation of the analyte and the IS was carried out using a Phenomenex Luna C18 column (30 × 2 mm, 3 μm) with water with 0.02% (v/v) formic acid: methanol as the mobile phase at a flow rate of 0.5 mL/min. ESI was used as an ion source. The analyte and IS were detected using SRM mode with m/z 60 and 246 for the analyte and 172 and 231 for the IS, respectively [51].

Ghosh *et al.* developed and validated an LC-ESI-MS method for the quantification of CLN in human plasma samples using carbamazepine as the IS. The analyte was extracted from plasma by LLE using ethyl acetate as the extracting solvent. The separation of the analyte and IS was carried out using an Inertsil ODS 3V C18 column (150 × 4.1 mm, 5 μm) with acetonitrile:0.1% (v/v) formic acid in water (90:10, v/v) as the mobile phase at a flow rate of 0.75 mL/min

and was detected using MRM mode. The m/z was 230 for the analyte and 194 and 237 for the IS, respectively [162]. Other LC-MS/MS methods for the quantification of CLN in human plasma samples [163-166] and their LC-MS chromatographic conditions are reported in Table 2.

Pelzer *et al.* developed and validated an LC-MS/MS method for the quantification of CLN extracted from human serum samples using CLN-D4 as the IS. The analyte and IS were separated using a Betasil silica column (50 × 3 mm, 5 μm) with acetonitrile:water:formic acid (80:20:1, v/v/v) as the mobile phase at a flow rate of 0.7 mL/min. ESI was used as an ion source. The analyte and IS were detected using MRM mode with m/z 213 and 230 for the analyte and 219 and 236 for the IS, respectively [54].

Liquid chromatography methods for the determination of enantiomers of AMP in biological fluids have been reported using different approaches: chiral derivatization, chiral stationary phase and chiral additives in the mobile phase [167,168]. Foster *et al.* developed an elution reverse mode HPLC method for the determination of AMP enantiomers present in human urine samples. The extraction of enantiomers from biological samples was done by SPE and the extracted enantiomers were derivatized with chiral Marfey's reagent to form diastereomers. The obtained diastereomers were separated using a C18 column (150 × 4.6 mm, 5 μm) with water:methanol (40:60, v/v) as the mobile phase at a flow rate of 1 mL/min with UV detection at 340 nm. The LOD of each enantiomer was found to be 0.16 mg/L [169].

Jose *et al.* developed an HPLC method for the determination of isomers of MPH in human plasma samples. The chromatographic separation was carried out using a Chirobiotic V2 column (150 × 4.6 mm, 5 μm) with methanol: 20 mM ammonium acetate, pH 4.1 (92:08, v/v) as the mobile phase at a flow rate of 1 mL/min. The *d* and *l*-isomers of MPH were retained at 7 and 8.1 min, respectively and were detected by DAD/UV detector at 215 nm. The proposed method was validated and found to be linear, accurate and precise [170].

Thomsen *et al.* developed an LC-MS/MS method for the determination of enantiomers of MPH and its metabolite extracted from human whole blood using rac-threo-MPH-D10 as the IS. The separation of the drug and IS were carried out using a Chiral-AGP column (100 × 4 mm, 5 μm) with 10 mM ammonium formate buffer:0.4% (v/v) isopropanol with formic acid (pH 5.4) as the mobile phase at a flow rate of 0.6 mL/min. The analyte and IS were ionised using ESI as an ion source. The ions of the analyte and IS were detected using MRM mode and the m/z was 56, 84 and 234.2 for the analyte and 93 and 244.2 for the IS, respectively [171]. Other LC-MS/MS methods for the quantification of MPH in human plasma samples [172-174] are reported in Table 2.

3.4.3 Gas chromatography (GC)

GC is an analytical method used to measure the various components in a sample. The components need to be volatile and thermostable for GC analysis. The principle of separation is adsorption for gas solid chromatography (GSC) and partition for gas liquid chromatography (GLC). When sample is introduced into the instrument, it enters into a gas stream and passes through a column for separation. The separated components are detected using various detectors including flame ionization detector (FID), electron capture detector (ECD) and nitrogen phosphorous detector (NPD) [175]. Analysis by GC requires derivatization with suitable derivatizing agent before analysis to change the properties of the analyte for better separation, detection and to improve method sensitivity [176].

Anggard et al. proposed a GLC method for the quantification of AMP extracted from blood, cerebrospinal fluid (CSF), tissues and urine samples. The extracted analyte from urine samples was derivatized by adding trifluoroacetic anhydride and separated using a glass column (3.04 m × 4 mm) with carrier gas at a flow rate of 40 mL/min and detected by FID. The column, injector and detector temperature were set at 175°C, 235°C, and 240°C, respectively. The extracted analyte from blood and CSF samples was derivatized by adding trichloroacetyl chloride and was separated using a glass column (1.82 m × 4 mm) with carrier gas at a flow rate of 90 mL/min and detected by ECD. The column, injector and detector temperature were set at 200°C, 230°C, and 210°C, respectively. The method was found to be sensitive and accurate [177].

Brien et al. developed a GC method for the determination of AMP in human biological fluids such as whole blood and urine samples and detected using FID. The drug extracted from biological fluids was derivatized with trifluoroacetic anhydride and was separated using a glass column (1.82 m × 6.35 mm) with helium and hydrogen as the carrier gas. The column, detector and inlet temperature were set at 140°C, 250°C, and 190°C, respectively and the flow rate was set to 50 mL/min (for helium) and 35 mL/min (for hydrogen). The proposed method was validated, and it was suitable for analysis [178].

Dugal et al. developed a GC method for the determination of un-metabolized MPH in human urine samples. The extracted analyte was derivatized with trifluoroacetic anhydride and the separation was carried out using a glass coiled column (1.22 m × 2 mm) with helium as the carrier gas at a flow rate of 30 mL/min. The column, injector and detector temperature were set at 140°C to 270°C, 250°C, 300°C, respectively and detection was using NPD [179]. Other GC methods for the quantification

of AMP and MPH in human biological samples [180-188] are reported in Table 3.

Guerret et al. reported a GLC method for the determination of GNF in human plasma and urine samples. The analyte was extracted and an excess amount of hexafluoro acetyl acetone and methanol was added for derivatization. The separation of the analyte was carried out using a glass column (1.6 m × 3 mm) packed with 3% OV-225 on 100-120-mesh Gas Chrom Q using 5% methane in argon as the carrier gas at a flow rate of 60 mL/min and detected by ECD. The injector, column and detector temperatures were set at 250°C, 210°C, and 300°C, respectively. The developed method was specific and sensitive [189].

Chu et al. developed a GC method to determine CLN and its derivatives extracted from human plasma and urine samples. The separation was carried out using a silanized glass column (1.8 m × 2 mm) with nitrogen as the carrier gas at a flow rate of 25 mL/min. The injector, column and detector temperatures were set at 220°C, 175°C, and 250°C, respectively and detection of the analyte was by ECD [190]. Edlund PO developed a GC method for the quantification of CLN extracted from human plasma samples and evaluated various types of columns, different carrier gases and altered the oven, injector and detector temperatures to develop a selective method for the analysis of CLN using ECD [191].

Kankaanpää et al. developed and validated a GC-MS method for the quantification of AMP in human blood, serum, oral fluids and urine samples. The analyte was extracted and derivatized with heptafluorobutyric anhydride (HFBA). The methylmexiletine was used as the IS. The analyte and IS were separated at retention times of 6.64 and 10.18 min, respectively using a DB-5MS column (30 m × 0.32 mm, 1 µm) with helium as the carrier gas and were detected using SIM mode. The m/z was found to be 118, 169, and 240 for the analyte and 136 and 254 for the IS, respectively. [192]. Miki et al. developed and validated a GC-MS method for the determination of AMP in human blood, hair and urine samples [193] and the chromatographic conditions are reported in Table 4.

Frison reported a validated GC-MS method for the determination of AMP in human plasma, hair and urine samples. The analyte was extracted and derivatized with 2, 2, 2-trichloroethyl chloroformate. 3, 4-methylenedioxypropylamphetamine (MDPA) was used as the IS. The analyte and IS were separated using a Hewlett-Packard HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm) with helium as the carrier gas at a flow rate of 1 mL/min and detected using SIM mode. The m/z was 162, 218, 220, 274, and 309 for the analyte and 260,

Table 3: GC methods for the determination of AMP and MPH in human biological samples.

Analyte	Biological samples	Stationary phase	Carrier gas	Flow rate	Injector Temperature	Column Temperature	Detector Temperature	Detector	Reference
AMP	Human blood & urine	Glass U-tube column (1.8 m × 4 mm)	Nitrogen	50 mL/min	195°C	160°C	185°C	FID	[180]
AMP	Human urine	AT-5 column (30 m × 0.25 mm, 0.25 µm)	Helium	1 mL/min	220°C	40°C to 280°C	280°C	FID	[181]
AMP	Human urine	DB5 capillary column (25 m × 0.32 mm, 0.25 µm)	Helium	2 mL/min	250°C	100°C to 260°C	270°C	FID	[182]
AMP	Human urine	BPX5 capillary column (30 m × 0.25 mm, 0.25 µm)	Helium	30 mL/min	280°C	30°C to 260°C	-	FID	[183]
MPH	Human blood & urine	Borosilicate glass column (1.52 m × 3 mm)	Nitrogen and hydrogen	75 mL/min	220°C	140°C	-	FID	[184]
MPH	Human urine	Glass column (1.82 m × 2 mm)	Nitrogen	25-30 mL/min	300°C	170°C	-	FID	[185]
AMP	Human hair	CBJ-17 capillary column (30 m × 0.53 mm, 1 µm)	Helium	4 mL/min	220°C	100°C to 220°C	220°C	NPD	[186]
MPH	Human plasma	Glass coiled column (1.83 m × 2 mm)	Helium	30 mL/min	240°C	170°C	270°C	NPD	[187]
AMP	Human plasma	Narrow bore fused silica capillary column (25 m × 0.32 mm, 1.05 µm)	Helium	2 mL/min	280°C	105°C	325°C	ECD	[188]

262, 264, 395, and 397 for the IS, respectively [194]. Other GC-MS methods for the quantification of AMP in human blood [195-198], plasma [199], serum [200], oral fluids [201,202], hair [203-206], and urine samples [207-210] are reported in Table 4.

Leis *et al.* reported a validated GC-MS method for the determination of MPH in human plasma samples using MPH-¹⁸O²H₃ as the IS. The analyte was extracted by LLE using *n*-hexane and derivatized with *o*-(pentafluorobenzoyloxycarbonyl)-2, 3, 4, 5-tetrafluorobenzoyl chloride. The derivatized analyte and IS were separated using a BPX5 fused silica capillary column (15 m × 0.25 mm) with helium as the carrier gas at a flow rate of 1.5 mL/min and detected using SIM mode. The *m/z* was found to be 408 and 452 for the analyte and 413 and 457 for the IS, respectively [211]. Further GC-MS methods for the quantification of MPH extracted from human plasma [212-214] and serum samples [215] are reported in Table 4.

Boumba *et al.* developed and validated a GC-MS method for the quantification of BUP in human whole blood, plasma and serum samples. The analyte was

extracted by SPE and was separated using an Equity 5 capillary column (30 m × 0.25 mm, 0.25 µm) with helium as the carrier gas at a flow rate of 1.5 mL/min and detected using SIM mode. The *m/z* of BUP was 44 [216].

Julien Larose *et al.* proposed a GC-MS method for the determination of GNF in human plasma samples using negative chemical ionisation. GNF-¹³C¹⁵N₃ was used as the IS and the analyte was derivatized with hexafluoro acetyl acetone. The analyte and IS were separated using a fused silica capillary column with helium as the carrier gas and were detected using SIM mode. The *m/z* was 417 for the analyte and 423 for the IS, respectively. The method was validated and was found to be useful for pharmacokinetic studies [217].

Haglock *et al.* developed and validated a GC-MS method for the determination of GNF in human urine samples. Protriptyline was used as the IS and the analyte was derivatized with HFBA. The analyte and IS were separated using a DB-5 capillary column (30 m × 0.32 mm, 0.25 µm) and were detected using SIM mode. The *m/z* was 86.1, 272.1, and 274.1 for the analyte and 189.1 and 191.1 for the IS, respectively [218].

Table 4: GC-MS methods for the determination of AMP, MPH and CLN in human biological samples.

Analyte	Derivatizing agent	IS	Stationary phase	Carrier gas	Flow rate	Detection	m/z	Reference
AMP	N-methyl bis(trifluoroacetamide)	Diphenyl-methane	DB-5MS or DB-1MS capillary column (30 m × 0.32 mm, 0.25 µm)	Helium	3 mL/min	SIM	140 (AMP) & 168 (IS)	[193]
AMP	Perfluoro octanoyl chloride	AMP-D3	Hewlett-Packard column (12 m × 0.2 mm, 0.33 µm)	Helium	1.4 mL/min	SIM	118, 440 (AMP) & 121, 443 (IS)	[195]
AMP	HFBA	AMP-D5	HP 5 MS capillary column (30 m × 0.32 mm, 0.25 µm)	Helium	-	SIM	91, 118 & 240 (AMP), 244 (IS)	[196]
AMP	Propyl-chloroformate	Methamphetamine-D5	Fused silica capillary column (30 m × 0.25 mm, 0.25 µm)	Helium	0.8 mL/min	SIM	130 (AMP) & 148 (IS)	[197]
AMP	S-(<i>-</i>)-hepta-fluorobutyl prolyl chloride	AMP-D11	HP-5MS column (30 m × 0.25 mm)	Helium	1 mL/min	SIM	388 (AMP) & 399 (IS)	[198]
AMP	HFBA	AMP-D6	Fused-silica DB-1 capillary column (30 m × 0.25 mm, 0.25 µm)	Helium	-	SIM	311.1 (AMP) & 317.1 (IS)	[199]
AMP	HFBA	Methamphetamine-D5	Fused-silica capillary (DB-5 MS) column (30 m × 0.25 mm, 0.25 µm)	Helium	1 mL/min	SIM	44, 91 & 135 (AMP), 62, 92 & 154 (IS)	[200]
AMP	Pentafluoro propionic acid	AMP-D5	Agilent HP-5MS column (30 m × 250 µm, 0.25 µm)	Helium	1 mL/min	SIM	91, 118 & 190 (AMP), 122, 123 & 194 (IS)	[201]
AMP	Propyl chloroformate	AMP-D5	HP-5MS column (30 m × 0.25 mm, 0.25 µm)	Helium	1 mL/min	SIM	130 (AMP) & 134 (IS)	[202]
AMP	Ethyl chloroformate	Methamphetamine-D5	Fused silica capillary column (30 m × 0.25 mm, 0.25 µm)	Helium	0.8 mL/min	SIM	116 (AMP) & 134 (IS)	[203]
AMP	Pentafluoro propionic anhydride-pentafluoro propanol.	AMP-D5	HP-5MS column (30 m × 0.25 mm, 0.25 µm)	Helium	1 mL/min	SIM	118 & 190, (AMP), 123 & 194 (IS)	[204]
AMP	Propionic acid anhydride	Methaqualone	HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm)	Helium	1 mL/min	SIM	44, 91, 100 & 118 (AMP), 235 & 250 (IS)	[205]
AMP	Heptafluoro butyric anhydride	AMP-D5	HP-5MS column (30 m × 0.25 mm)	Helium	1 mL/min	SIM	240 (AMP) & 244 (IS)	[206]
AMP	Alkyl chloroformates	Methamphetamine-D5	Fused-silica capillary column (30 m × 0.25 mm, 0.25 µm)	Helium	0.8 mL/min	SIM	91, 116, 207 (AMP) & 134 (IS)	[207]
AMP	Bis(trimethylsilyl)-trifluoro-acetamide and trimethyl chlorosilane	AMP-D5	HP-5 fused-silica capillary column (30 m × 0.25 mm, 0.32 µm)	Helium	1 mL/min	SIM	91, 116 & 192 (AMP), 92, 120 & 197 (IS)	[208]

Analyte	Derivatizing agent	IS	Stationary phase	Carrier gas	Flow rate	Detection	m/z	Reference
AMP	Acetic anhydride	-	Restek Rtx-5MS capillary column (30 m × 0.25 mm, 0.25 µm)	Helium	0.8 mL/min	SIM	44 (AMP)	[209]
AMP	Pentafluoro-propionic anhydride	AMP-D11	DB-5MS column (30 m × 0.25 mm, 0.25 µm)	Helium	-	SIM	190 (AMP) & 194 (IS)	[210]
MPH	HFBA	MPH- 18O2	Restek Rtx-5MS fused-silica capillary column (15 m × 0.25 mm, 0.25 µm)	Helium	1.5 mL/min	SIM	369 (MPH) & 373 (IS)	[212]
MPH	o-(pentafluoro- benzyloxy carbonyl)-benzoyl chloride	MPH- 18O2H3	BPX5 fused silica capillary column (15 m × 0.25 mm)	Helium	1.5 mL/min	SIM	380 (MPH) & 385 (IS)	[213]
MPH	N-Heptafluoro butyryl-L-prolyl chloride	threo - ethylphenidate. HCl	Silanized glass column (1 m × 2.6 mm)	Helium	37 mL/min	SIM	527 (MPH) & 541 (IS)	[214]
MPH	Trifluoro acetic anhydride	Ethylphenidate	Glass, coiled column (2 m × 2 mm)	Helium	20 mL/min	SIM	180 (MPH & IS)	[215]
CLN	3, 5-Bis (tri-fluoromethyl) benzoyl chloride	CLN-2H4	Fused-silica capillary column (25 m × 0.23 mm, 0.13 µm)	Helium	-	SIM	673 & 709 (CLN), 676 & 713 (IS)	[220]
CLN	Pentafluoro benzyl bromide	Moxonidine	DB 5 capillary column (30 m × 0.25 mm, 0.25 µm)	-	-	SIM	354 (CLN) & 366 (IS)	[221]

Girault et al. developed and validated a GC-MS method for the determination of CLN extracted from human plasma samples. 2- [(2, 4-dichlorophenyl) imino] - imidazolidine was used as an IS and the analyte was derivatized with pentafluorobenzyl bromide. The analyte and IS were separated using an Ultra 1 bonded phase fused-silica capillary column (25 m × 0.2 mm, 0.33 μm) with helium as the carrier gas and detected using SIM mode. The m/z was 354 for the analyte and IS [219]. Other GC-MS methods for the quantification of CLN extracted from human plasma [220] and serum [221] samples are reported in Table 4.

Woźniak et al. developed and validated a GC-MS/MS method for the quantification of AMP in human blood and urine samples using metamphetamine-D5 as the IS. The analyte was extracted from biological samples by LLE using ethyl acetate as the extracting solvent and derivatized with trifluoroacetic anhydride. The analyte and IS were separated using a Phenomenex ZB-5MSi capillary column (30 m × 0.25 mm, 0.25 μm) with helium as the carrier gas at a flow rate of 1 mL/min and detected using MRM mode. The m/z value was 68.9, 91, 118, and 140 for the analyte and 113 and 158 for the IS [222].

Gambelunghé et al. proposed a GC-MS/MS method for the determination of AMP in human hair samples using AMP-D6 as the IS. The analyte was extracted and derivatized with pentafluoro propionic anhydride for analysis. The analyte and IS were separated using a CP-SIL

8CB-MS capillary column (30 m × 0.25 mm, 0.25 μm) with helium as the carrier gas at a flow rate of 1 mL/min and detected using SIM mode. The m/z was 148 for the analyte and 194 for the IS, respectively [223].

Lachenmeier et al. reported a head space GC-MS/MS method for the quantification of AMP in human hair samples using AMP-D5 as the IS and a triple quadrupole as the mass analyser. A solid-phase dynamic extraction technique was employed for sample preparation. The analyte and IS were separated using a fused-silica capillary column (30 m × 0.25 mm, 0.25 μm) with helium as the carrier gas at a flow rate of 1 mL/min. The analyte and IS were detected using both MRM and SIM mode and the m/z were 93 and 140 for the analyte and 96 and 144 for the IS, respectively. The method was validated and was useful for clinical and forensic studies [224]. There is no reported GC method for the quantification of ATX in human biological samples.

4 Discussion

This review covers almost all of the analytical methods for the determination of ADHD drugs in human biological matrices from the year 1970 to 2019. A greater number of analytical methods were reported for stimulant drugs i.e. AMP and MPH compared to non-stimulant drugs i.e. ATX, BUP, GNF, and CLN, which is represented in Figure 2.

Figure 1: Structures of ADHD drugs.

Figure 2: Availability of reported analytical methods for ADHD drugs.

Figure 3: Various bio-analytical methods used for quantification of ADHD drugs.

In general, spectrophotometry and spectrofluorimetry are the simplest and most inexpensive techniques used for the quantification of drugs. In bioanalysis, a limited number of these methods were reported for the quantification of ADHD drugs, as it detects analytes at the level of $\mu\text{g/mL}$. For spectrophotometric analysis, addition of a chromogenic reagent (NQS) is required for the analysis of AMP with higher molar absorptivity. The analytes like BUP and GNF are basically non-fluorescent compounds, by reaction with reagents such as dansyl chloride, fluorescent compounds are produced which emit fluorescence at certain wavelengths and are measured using fluorimetry. Nowadays, hyphenated techniques are used for an advanced level of analysis and these have greater applications in the field of bioanalysis due to improved selectivity, sensitivity, accuracy and precision. The various

bio-analytical methods used for the quantification of ADHD drugs in biological samples are overviewed in Figure 3. It is found that a greater number of LC-MS/MS methods were reported for the quantification of the six ADHD drugs. Compared to other LC detectors (UV-Vis, fluorescence, etc.), MS is more significant in terms of selectivity and sensitivity. LC-MS/MS methods in bioanalysis significantly reduce the LC run time and increase the sample throughput. To give high quality bioanalytical data, LC-MS/MS is the preferred method for the fast and sensitive quantification of ADHD drugs in biological matrices with a lower detection limit (ng to pg/mL).

5 Conclusion

In this review, the chemistry, physio-chemical properties and bio-analytical methods of six ADHD drugs were discussed. The stimulant drugs (short acting) are the most commonly prescribed drugs compared to non-stimulant drugs in the treatment of ADHD in children and adolescents. Thus, various bio-analytical methods were developed and validated for quantification of these drugs in biological matrices and were applied to pharmacokinetic, toxico-kinetic studies, etc. From the above discussion, we concluded that LC-MS/MS is well-developed and the most suitable method for the quantification of ADHD drugs in human biological samples.

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