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REVIEW PAPER

Application of LC–MS/MS for quantitative analysis of glucocorticoids and stimulants in biological fluids

Jamshed Haneef[a], Mohammad Shaharyar[a], Asif Husain[a], Mohd Rashid[a,*], Ravinesh Mishra[a], Shama Parveen[a], Niyaz Ahmed[a], Manoj Pal[b], Deepak Kumar[c]

[a]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi 110062, India*
[b]*Department of Chemistry, Indian Institute of Technology (IIT), Haridwar, Roorkee 247667, Uttarakhand, India*
[c]*Research Institute of Natural Sciences, Department of Chemistry, Changwon National University, Changwon 641773, Republic of Korea*

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Abstract Liquid chromatography tandem mass chromatography (LC–MS/MS) is an important hyphenated technique for quantitative analysis of drugs in biological fluids. Because of high sensitivity and selectivity, LC–MS/MS has been used for pharmacokinetic studies, metabolites identification in the plasma and urine. This manuscript gives comprehensive analytical review, focusing on chromatographic separation approaches (column packing materials, column length and mobile phase) as well as different acquisition modes (SIM, MRM) for quantitative analysis of glucocorticoids and stimulants. This review is not meant to be exhaustive but rather to provide a general overview for detection and confirmation of target drugs using LC–MS/MS and thus useful in the doping analysis, toxicological studies as well as in pharmaceutical analysis.

1. Introduction

Corticosteroids and stimulants are the class of compounds that are illicitly used by professionals owing to their anti-inflammatory and mood elevating as well as euphoric properties respectively. They are widespread abused among sports persons [1], stimulant addiction among teenagers and deliberate counterfeiting in herbal products to enhance their effects [2,3]. Hence there is a need for more sensitive analytical tools to detect and confirm these classes of drugs in biological fluids [3,4]. These analytical tools would serve to fulfill demands in forensic, toxicological and food safety departments. Glucocorticoid belongs to steroid family, particularly

*Corresponding author. Tel.: +91 11 26059681, +91 11 26059688x5647, mobile: +91 9911609781; fax: +91 11 16988874.
E-mail addresses: rashidpharm2008@gmail.com, rashidchem2012@gmail.com (M. Rashid)

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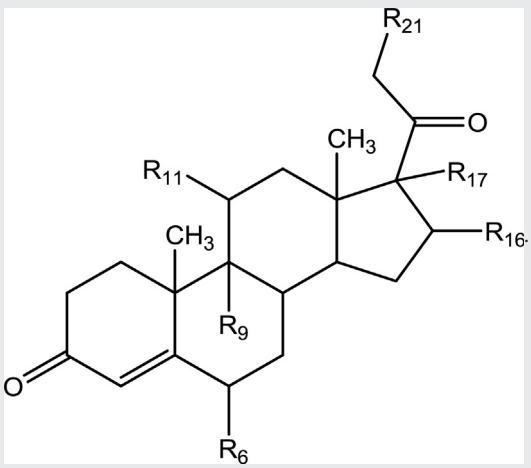
of pregnane class containing C-21 derivatives. Glucocorticoids have important functions upon carbohydrate, protein and calcium metabolism, potent anti-inflammatory and immunosuppressive activities [5–8]. The activity of glucocorticoids largely depends upon the substituent attached to the nucleus. Substituent has been found to significantly increase both glucocorticoids and mineralocorticoids activities. It has been found that $\Delta^{1,2}$ corticosteroids enhance anti-inflammatory activity and decrease salt retaining activity [9]. Some selected glucocorticoids and their structures are shown in Table 1. Stimulants are the class of drugs that have a marked effect on mental function and behavior, producing euphoria and reduced fatigue. They are diverse class of compounds that exhibit their action through different mechanism. This class of drugs is often abused by teenagers leading to addiction and risk of health hazards. Some of selected stimulants are modafinil, famprofazone, tuaminoheptane, amiphenazole, amphetamine, methamphetamine, dimethylamphetamine, methylphenidate, 3,4-methylene-dioxy-N-amphetamine, 3,4-methylene-dioxy-N-ethylamphetamine, strychnine and 3,4-methylene-dioxy-N-methamphetamine etc.

Thus there is need for sophisticated and robust analytical strategy to confirm their presence in biological fluids. A number of different analytical approaches are available for this purpose. Analytical techniques such as high performance liquid chromatography (HPLC), ultra high performance liquid chromatography (UHPLC), mass spectrometry, gas chromatography, electrochemical detection and enhanced confirmatory procedures are used in detection of these classes of drugs. Recent development and advancement in analytical technologies has emerged with more sophisticated hyphenated techniques, to detect nanograms of drugs or their metabolites in biological fluids. Among hyphenated techniques, LC–MS/MS is the choice of interest because it is highly sophisticated and considerably powerful tool for detection of low and high molecular weight analytes. New methods have enabled the determination of drugs that were formerly difficult to detect by conventional methods of analysis as well as time consuming procedures have been replaced by faster, more comprehensive and robust assays. Good sensitivity and high throughput are key factors for the LC–MS/MS approaches used in drug analysis.

In this review, we present an overview of the methodologies that are reported in literature for detection, confirmation of corticosteroids and stimulants in biological fluids. Reported screening methods using LC–MS/MS approach highlight chromatographic separation and different modes of instrumental acquisition (selective ion monitoring (SIM), multiple reaction monitoring (MRM), precursor ion scan) for

Table 1 General nucleus of corticoids and different substitutions of different glucocorticoids drugs.



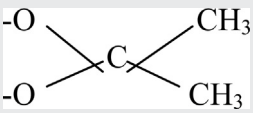
Steroid name	$\Delta^{1,2}$	R_6	R_9	R_{11}	R_{16}	R_{17}	R_{21}
Hydrocortisone	Saturated	–H	–H	—OH	–H	—OH	—OH
Cortisone	Saturated	–H	–H	=O	–H	—OH	—OH
Corticosterone	Saturated	–H	–H	—OH	–H	–H	—OH
Fludrocortisone	Saturated	–H	–F	—OH	–H	—OH	—OH
Prednisolone	Double bond	–H	–H	—OH	–H	—OH	—OH
Prednisone	Double bond	–H	–H	=O	–H	—OH	—OH
Methylprednisolone	Double bond	—CH_3	–H	—OH	–H	—OH	—OH
Prednisolone acetate	Double bond	–H	–H	—OH	–H	—OH	—$OCOCH_3$
Dexamethasone	Double bond	–H	–F	—OH	CH_3 (á isomer)	—OH	—OH
Betamethasone	Double bond	–H	–F	—OH	CH_3 (â isomer)	—OH	—OH
Beclomethasone	Double bond	–H	–Cl	—OH	CH_3 (â isomer)	—OH	—OH
Triamcinolone	Double bond	–H	–F	—OH	—OH	—OH	—OH
Triamcinolone acetonide	Double bond	–H	–F	—OH			—OH

Table 2 Selected confirmatory methods for detecting glucocorticoids in biological fluids.

Analyte	Matrix	Pretreatment	Column; mobile phase	Detection mode	LOD	Ref.
9 Synthetic corticosteroids	Urine	LLE with Extrelut-NT3 columns	Inertsil ODS-3 C18 column (150 mm × 3 mm, 3 μm); 1 mM ammonium acetate pH 6.8/ACN (60:40, v/v) at 0.4 mL/min	ESI negative SIM mode	1 ng/mL	[12]
20 Corticosteroids	Urine	Enzymatic hydrolysis C-8 SCX SPE Cartridge	Supelcosil LC-8-DBcolumn (2.1 mm × 3.3 cm, 3 μm); 5 mM acetic acid/10 mM ammonium acetate (pH 3.8)/ methanol, at 0.2 mL/min	ESI positive MRM	0.1–10 ng/mL	[13]
Methylprednisolone methylprednisolone acetate	Plasma	Protein precipitation	Sinergy Max RP C-12 column (2.0 mm × 150 mm, 4 μm); 0.01% formic acid in water/ACN (50:50, v/v) at 0.2 mL/min	ESI positive MRM	6–300 ng/mL	[14]
17 Synthetic glucocorticoids	Urine	Enzymatic hydrolysis followed by LLE	BEH C18 column(2.1 mm × 50 mm, 1.7 μm); 0.1% formic acid in ACN/0.1% formic acid in water, at 0.35 mL/min	TOF-MS with ESI positive	0.1–3.3 μg/L	[15]
Methylprednisolone	Methylprednisolone Plasma	LLE	Pursuit C-18 column (2.0 mm × 150 mm, 5 μm); ACN/0.5% formic acid aq. solution (85:15, v/v) at 0.2 mL/min	ESI positive SRM	20–2000 ng/ mL	[16]
5 Corticosteroids: dexamethasone, flumethasone, fluorometholone, beclomethasone, triamcinolone acetonide	Urine	Enzymatic hydrolysis Oasis-HLB SPE column	Alltima C-18 column (2.1 mm × 150 mm, 5 μm); ACN/water(40:60, v/v) at 0.3 mL/min	APCI positive SRM mode	2–3 ng/mL	[17]
Cortisol, cortisone, prednisolone, prednisone	Plasma	Protein precipitation	Zorbax-SB phenyl, HT rapid resolution column (2.1 mm × 100 mm, 1.8 μm); ACN/H_2O/ formic acid(32:68:0.1, v/v) at 0.140 mL/min	APCI positive MRM mode	0.5–2 ng/mL	[18]
Prednisone, prednisolone, dexamethasone, cortisol	Human serum	SPE	Symmetry C-18 column (2.1 mm × 30 mm, 3.5 μm); methanol/ 5 mM acetate buffer pH 3.25 at 400 μL/ min	ESI negative MRM mode	0.2–0.5 ng/mL	[19]
Cortisol, dexamethasone, prednisone, prednisolone, methylprednisolone	Plasma	SPE Oasis HLB Cartridge	Symmetry C-18 column (2.1 mm × 30 mm, 3.5 μm); 5 mM ammonium acetate buffer pH 3.5 and methanol (95:5, v/v)/methanol and 5 mM ammonium acetate buffer pH 3.5 (95:5, v/v), at 400 μL/min	ESI negative MRM mode	3–7 ng/mL	[20]
21 Synthetic corticosteroids	Urine	Enzymatic hydrolysis followed by LLE	RP DB-8 column (4.6 mm × 75 mm, 3 μm); 1% acetic acid/ methanol, at 1 mL/min	APCI positive full scan mode	5 ng/mL	[21]
Cortisol, cortisone, prednisolone, dexamethasone, 11-deoxycortisol	Plasma, urine saliva, plasma-ultra filtrate	SPE Oasis HLB 1 cm^3 Cartridge	Acquity UPLC BEH C-18 column (2.1 mm × 50 mm, 1.7 μm); $A=2$ mM ammonium acetate in water with 0.1% formic acid, $B=2$ mM ammonium acetate in methanol with 0.1% formic acid, at 0.4 mL/min, total run time 3 min.	ESI positive MRM	0.6–5 nM	[22]
16 Glucocorticoids	Urine	Enzymatic hydrolysis followed by LLE	Halo C-18 column(2.1 mm × 150 mm, 2.7 μm and 2.1 mm × 100 mm, 2.7 μm); water with 0.1% formic acid/ACN with 0.1% formic acid at 400 μL/min	ESI positive MRM	5–15 ng/mL	[23]
15 Synthetic glucocorticoids	Urine	Enzymatic hydrolysis followed by LLE	Zorbax C-18 column (2.1 mm × 50 mm, 1.8 μm); 0.1% acetic acid/ACN with 0.1% acetic acid at 0.3 mL/min	ESI positive MRM	1–30 ng/mL	[24]
14 Glucocorticoids	Urine	Enzymatic hydrolysis followed by LLE	Inertsil ODS-3 C18 column (50 mm × 4.6 mm, 3 μm); 1% formic acid/ACN, at 700 μL/min	ESI positive MRM	1–5 ng/mL	[25]

Ref.—References.

Table 3 Selected confirmatory methods for detecting stimulants in biological fluids.

Analyte	Matrix	Pretreatment	Column; Mobile phase	Detection mode	LOD	Ref.
9 Stimulants including strychnine, methylphenidate, amiphenazole, famprofazone, tuaminoheptan	Urine	Enzymatic hydrolysis followed by LLE	Halo C-18 column (2.1 mm × 150 mm, 2.7 µm and 2.1 mm × 100 mm, 2.7 µm); water with 0.1% formic acid/ACN with 0.1% formic acid at 400 µL/min	ESI positive MRM	70–300 ng/mL	[23]
Mesocarb, modafinil, formoterol	Urine	Enzymatic hydrolysis followed by LLE	Zorbax C-18 column (2.1 mm × 50 mm,1.8 µm); 0.1% acetic acid/ACN with 0.1% acetic acid at 0.3 mL/min	ESI positive MRM	100–200 ng/mL	[24]
Methylphenidate, mesocarb	Urine	Enzymatic hydrolysis followed by LLE	Inertsil ODS-3 C18 column (50 mm × 4.6 mm, 3 µm); 1% formic acid/ACN, at 700 µL/min	ESI positive MRM	5 ng/mL	[25]
49 Stimulants	Urine	Enzymatic hydrolysis SPE cartridge	Phenomex Luna C-18 column(2 mm × 100 mm, 3 µm); 5 mM ammonium acetate with 0.1% formic acid/ ACN, at 0.3 mL/min	ESI positive	0.025 µg/mL	[26]
27 Amphetamine and amphetamine like drugs	Urine	LLE	Omnispher C-18 column (3.0 mm × 50 mm, 3 µm); 0.1% formic acid/methanol, at 0.4 mL/min	APCI positive full scan MS	1–25 ng/mL	[27]
7 Amphetamine derivatives: AP, MA, MDA, MDMA, MDEA, DMA, DMANO	Urine	Oasis HLB SPE	Capcell C-18 MG-II column (2.0 mm × 150 mm, 5 µm); 5 mM ammonium formate pH 4.0/ACN, at 230 µL/min	ESI positive MRM	1.95 ng/mL	[28]
Mesocarb	Urine	Enzymatic hydrolysis followed by LLE	Thermo-Hyper Gold C-18 (2.1 mm × 50 mm, 3 µm); 15 mM ammonium formate containing 1% formic acid/ACN at 200 µL/min	SRM positive	5 ng/mL	[29]
AP, MA, MDA, MDMA, MDEA, PMA, ephedrine	Urine	LLE	Acquity UPLC HSS C-18 column (2.1 mm × 100 mm, 1.8 µm); 5 mM ammonium formate buffer containing 0.05% formic acid/ methanol, at 0.3 mL/min	ESI positive MRM	0.5–2.5 ng/mL	[30]

AP: amphetamine, MA: methamphetamine, MDA: 3,4-methylenedioxy-N-amphetamine, DMA: N, N-dimethylamphetamine, PMA: p-hydroxy-methamphetamine, MDMA: 3,4-methylenedioxy-N-methamphetamine, MDEA: 3,4-methylenedioxy-N-ethylamphetamine, DMANO: N, N-dimethyl amphetamine-N-oxide, SPE: solid phase extraction, LLE: liquid–liquid extraction, ACN: acetonitrile, Ref: references.

Table 4 Empirical formula, exact masses, MS fragmentation data, Log *P* and λ_{max} of selected glucocorticoids and stimulants.

Drug name	Empirical formula	Exact mass	$[M+H]^+$	m/z of major fragments reported	Log *P*[a]	λ_{max} (nm)	Ref.
Amphetamine	$C_9H_{13}N$	135.10480	136.2	119.2, 91.2	1.8	257, 263	[28]
MDA	$C_{10}H_{13}NO_2$	179.09463	180.2	163, 134.9, 105	1.64	233, 285	[28,30]
MDMA	$C_{11}H_{15}NO_2$	193.11028	194.1	163.2, 105.1	2.15	234, 285	[28,30]
Beclomethasone	$C_{22}H_{29}ClO_5$	408.17035	409.18	391, 373, 337	2.03	239	[17,24]
Betamethasone	$C_{22}H_{29}FO_5$	392.19990	393	337, 355, 241, 147	1.94	240	[24,25,35–37]
Budesonide	$C_{25}H_{34}O_6$	430.23554	431	413, 341, 323, 173	2.18	248	[24,32,36,37]
Dexamethasone	$C_{22}H_{29}FO_5$	392.19990	393	373, 355, 337, 237, 147	1.83	240	[17,24,35–37]
Fludrocortisone	$C_{21}H_{29}FO_5$	380.19990	381	343, 239	1.67	240	[24,36]
Flumethasone	$C_{22}H_{28}F_2O_5$	410.19047	411	371, 335, 253, 121	1.9	238	[17,24,32,35]
Fluocortolone	$C_{22}H_{29}FO_4$	376.20499	377	321, 303	2.1	242	[24]
Methylphenidate	$C_{14}H_{19}NO_2$	233.14158	234	174, 129, 84, 56	0.20	264	[23,25,35]
Methylprednisolone	$C_{22}H_{30}O_5$	374.20932	375	357, 339, 161	1.82	240	[16,25,36,37]
Prednisolone	$C_{21}H_{28}O_5$	360.19367	361	343, 325, 147, 307	1.62	240	[24,25,35–37]
Prednisone	$C_{21}H_{26}O_5$	358.17802	359	341, 323, 267, 147, 34	1.46	240	[24,25,35–37]
Strychnine	$C_{21}H_{22}N_2O_2$	334.16813	335	264, 184, 156	1.9	255	[21,23]
Triamcinolone	$C_{21}H_{27}FO_6$	394.17917	395	357, 321, 225	1.16	238	[24,25,36,37]
Triamcinolone Acetonide	$C_{24}H_{31}FO_6$	434.21047	435	415, 397, 213	1.2	238	[24,25,36,37]

[a]Values obtained from online SRC Phys Prop Database, Ref.—References.

Table 5 Characteristics fragment ions of glucocorticoids obtained in ESI negative mode LC–MS/MS.

Analyte	*m/z* fragments
Cortisone	329.2[a], 301.2, 311.2
Dexamethasone	361.2[a], 307.2, 325.2
Hydrocortisone	331.2[a], 297.2, 282.2
Flumethasone	379.2[a], 325.2, 305.2
Flunisolide	375.2[a], 357.2, 433.2
Methylprednisolone	343.2[a], 309.2, 294.2
Prednisolone	329.2[a], 295.2, 280.2
Prednisone	327.2[a], 299.2, 285.2
Triamcinolone	345.2[a], 325.2, 393.2
Triamcinolone acetonide	413.2[a], 337.2, 375.2, 469.2

[a]Indicate base peak.

MS detection. Proposed mass fragmentation pathways of selected drugs are incorporated in this review.

2. Screening methods for detection of glucocorticoids and stimulants in biological fluids using liquid chromatography mass spectrometry

LC–MS/MS techniques provide specific, selective and sensitive quantitative results with reduced sample preparation. Other techniques such as electrochemical detection were also explored for the analysis of drugs. Goyal et al. [10,11] investigated electrochemical behavior of dexamethasone and triamcinolone at the fullerene-C60-modified pyrolytic graphite electrode (PGE) using Osteryoung square wave voltammetry (SWV) and they illustrated quantitative determination of dexamethasone and triamcinolone in several commercially available pharmaceutical formulations and human blood plasma of patients being treated with dexamethasone. LC–MS/MS is currently most ideal tools for screening and quantifying corticosteroids in biological fluids as compared to other conventional techniques. This technique is widely used for pharmacokinetic (PK) studies, metabolites identification in plasma and urine, doping analysis and forensic studies. The introduction of commercial hyphenated instruments in which liquid chromatography is coupled with different mass analyzers such as time of flight mass spectrometry (LC–TOFMS), triple quadrupole mass spectrometer and soft ionization techniques [electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), atmospheric pressure photo ionization (APPI), and matrix assisted laser desorption ionization (MALDI) etc.] has simplified the laboratory analysis and also decreased the cost of analysis to some extent. The polarity and functionalities of corticosteroids and stimulants allow the use of ionization techniques in positive ion or negative ion modes and different modes of instrumental acquisition for mass detection. Tables 2 and 3 summarize the information on methods used for identification and confirmation of corticosteroids [12–25] and selected stimulants [26–30].

Fluri et al. [12] reported confirmatory method for 11 corticosteroids in urine samples based on LC–ESI–MS. The selective and sensitive method for confirmation and identification of nine synthetic corticosteroids assured the exclusion of false positive results obtained by corticosteroid group ELISA screening tests. Emmie et al. [13] developed two high throughput LC–MS methods for the screening of anabolic steroids, corticosteroids, and acidic drugs using a high efficiency LC column coupled with a fast scanning triple quadrupole mass spectrometer. The detection of 40 anabolic steroids, corticosteroids, and 52 acidic drugs were achieved within a 6.5 min and a 4.5 min LC–MRM run, respectively and all the targets were detected at low amount. Validation data showed that both methods have acceptable precision to be used on a routine basis and no interference from sample matrix was observed.

A rapid, sensitive and specific method for the simultaneous detection and quantization of methylprednisolone acetate (MPA) and methylprednisolone (MP) in rat plasma, using a triple stage quadrupole was developed and validated by Panusa et al. [14] using LC–ESI–MS/MS. Its excellent applicability in PK studies was demonstrated. It was proved to be highly sensitive, allowing detection and quantization of the analytes at lower concentrations.

Method was developed by Touber et al. [15] using the latest high-resolution LC column technology, UPLC™, and ESI interface in the positive ion mode. Gradient UPLC separation conditions were optimized for a group of 22 analytes including 17 glucocorticosteroids. The UPLC–TOF–MS separation obtained required 5.5 min only for all the substances tested. Even the critical pair of dexamethasone and betamethasone isomers was almost completely resolved. The authors recommended that dedicated UPLC–TOF–MS criteria regarding the number of identification points (IPs), mass accuracy of parent, fragment ions, ion ratio, and relative retention time have not been assessed, in order to allow application of this new technology for confirmation of identity as well, that should be considered and thus needs improvement.

Mazzarino et al. [23] performed screening of several classes of substances in a single chromatographic method with a run-time of 11 min, inclusive of post-run and reconditioning times. The effectiveness of this approach was evaluated by LC–ESI–MS/MS in the positive mode, using 20 blank urine samples spiked with 45 compounds prohibited in sport including 16 glucocorticoids and 9 stimulants. All of the analytes were clearly distinguishable in urine, with limits of detection ranging from 5 ng/mL to 350 ng/mL. All the compounds of interest were separated, including synthetic and endogenous glucocorticoids with similar retention times and fragmentation patterns.

Kolmonen et al. [26] developed a general screening method based on solid phase extraction (SPE), LC–TOF/MS and validated 124 different doping agents including stimulants in urine. The result indicated that compared with conventional doping control methods, this method was more flexibility in identification, database management and reduced the time required for analysis.

A selective and sensitive method for the qualitative screening of urine samples for 27 amphetamine and amphetamine-type drugs was described by Deventer et al. [27] using mass spectrometer equipped with APCI interface, operated in positive ionization mode. They reported that the amount of urine routinely used in their laboratory for the extraction of these stimulants (5 mL) was reduced to 2 mL and thus reduced sample volume. The detection limits for all the compounds were lower than 25 ng/mL except for chlorphentermine, thus it was good alternative to gas chromatography with nitrogen phosphorus detector (GC-NPD).

Kim et al. [28] developed and validated LC–ESI–MS/MS method for the simultaneous detection and quantification of seven amphetamine derivatives amphetamine (AP), methamphetamine (MA), 3,4-methylenedioxy-N-amphetamine (MDA), 3,4-methylenedioxy-*N*-methamphetamine (MDMA), 3,4-methylenedioxy-N-ethylamphetamine (MDEA), N,N-dimethylamphetamine (DMA) and N,N-dimethylamphetamine-N-oxide (DMANO) in human

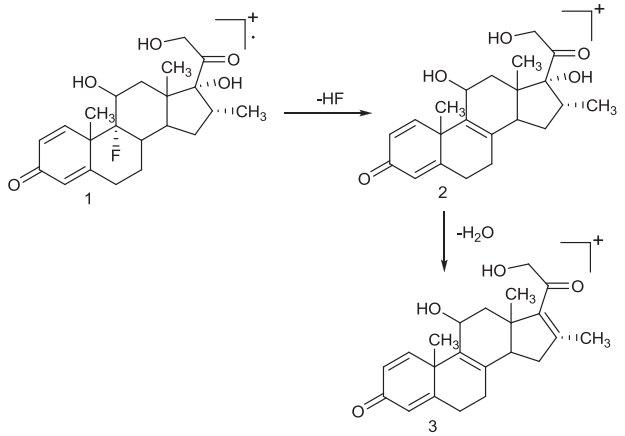


Fig. 1 Proposed mass fragmentation pathway of dexamethasone drug. 1—Molecular ion peak at $M^+=393$. 2—Base ion peak at $m/z=373$. 3—Daughter ion peak at $m/z=355$.

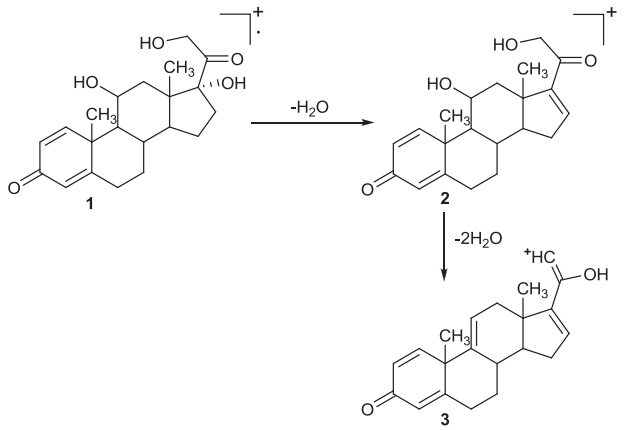


Fig. 2 Proposed mass fragmentation pathway of prednisolone drug. 1—Molecular ion peak at $M^+=361$. 2—Base ion peak at $m/z=343$. 3—Daughter ion peak at $m/z=307$.

urine. The paper reported that the SPE step was assayed to detect and quantify seven target analytes in urine samples without any significant interference from the matrix components.

Counterfeiting of herbal drugs with synthetic agents can also be detected by LC–MS/MS [31,32]. Different mass analyzers (TOF, IT (ion trap)) have been coupled with LC and extensively used for screening and characterization of different analytes in plasma and herbal extracts [33,34].

3. Focusing on different strategies for detection of glucocorticoids and stimulants in biological fluids

Based on the reported MS-fragmentation data, a common fragmentation could be developed in screening of corticosteroids and stimulants. The basic information regarding empirical formula, exact masses, Log *P* and absorption maxima (λ_{max}) is also summarized in Table 4. *P* and Log *P* are partition coefficient or logarithm of the partition coefficient of a drug. These parameters express the relative distribution of drug between oil and water under specified conditions for example, octanol/water at 37 °C and

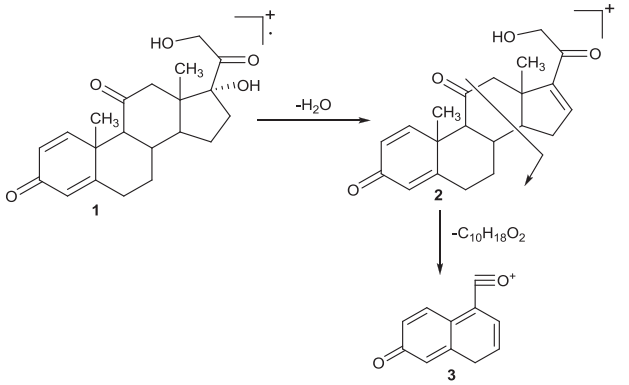


Fig. 3 Proposed mass fragmentation pathway of prednisone drug. 1—Molecular ion peak at $M^+=359$. 2—Base ion peak at $m/z=341$. 3—Daughter ion peak at $m/z=171$.

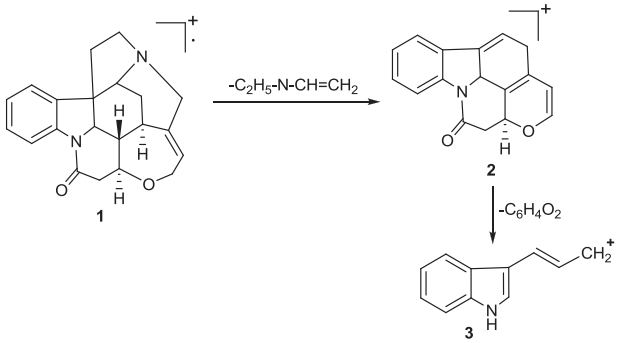


Fig. 4 Proposed mass fragmentation pathway of strychnine drug. 1—Molecular ion peak at $M^+=335$. 2—Base ion peak at $m/z=264$. 3—Daughter ion peak at $m/z=156$.

pH 7.4. Drugs with higher *P* or Log *P* are more lipophilic, generally distribute more rapidly and to a greater degree into bodily tissues and fluids. These physicochemical properties help in prediction of drug transport. These data render to presume interaction of analyte with stationary phase, thereby allowing a good evaluation of its chromatographic performance. UV spectra can help as a preliminary screening to distinguish different analytes on the basis of their absorption maxima (λ_{max}). The super-imposable nature of UV spectra of an analyte with a certified reference material (CRM) can give fair idea about their structural similarity to much extent. This approach has been employed for detection of adulteration using HPLC with UV detector.

The precursor and products ions summarized in Table 4, are based on positive ionization. Depending upon the nature of target analytes, ionization mode is optimized. Fluri et al. [12] studied both negative and positive ionization modes for examined glucocorticoids. Due to less fragmentation and a better signal-to-noise ratio (4:1), sensitivity increased for measurements in the negative ionization mode. They assessed that fragmentation of corticosteroids in the ESI negative mode is simple as few ions were produced. The products ions of ten selected corticosteroids by their study are presented in Table 5.

4. Proposed mass fragmentation pattern

The hypothetical mass fragmentation pattern of some selected drugs like dexamethasone, prednisolone, prednisone and

strychnine on the basis of MRM transitions has been incorporated and that helps in confirmation of analytes on the basis of diagnostic ions (Figs. 1–4). The daughter ions generated from the parent ions help to predict the fragmentation pattern of the molecule and are useful in confirmation of the target analytes.

5. Conclusion

With the advancement of hyphenated techniques, high resolution mass analyzers as well as high throughput separation approaches, quantitative analysis of glucocorticoids and stimulants can be achieved with good sensitivity. Newer methods can be developed for routine analysis of target analytes in biological fluids with shorter run time and good detectability. Application of new fused core columns for their effectiveness and use of both positive and negative polarities in a single run need to explore.

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