Research Article

Drug Testing and Analysis

Received: 30 June 2009 Revised: 22 September 2009 Accepted: 23 September 2009 Published online in Wiley Interscience:

(www.drugtestinganalysis.com) DOI 10.1002/dta.75

Detection and characterization of synthetic steroidal and non-steroidal anti-inflammatory drugs in Indian ayurvedic/herbal products using LC-MS/TOF

Akash A. Savaliya, Bhagwat Prasad, Dhara K. Raijada and Saranjit Singh*

It is claimed that ayurvedic/herbal healthcare products (AHPs) are safe because of their natural origin. However, several reports exist of adulteration of AHPs with synthetic drugs. In this study, a generalized strategy was developed using LC-MS/TOF for the detection and verification of steroidal and anti-inflammatory drugs in 58 AHPs collected from various parts of India. The strategy involved recording of mass spectral information for standard drugs – including ionization mode (ESI/APCI −ve or +ve), mass spectrum, accurate mass, identification of qualifier fragments (two), extracted ion chromatograms (EICs), isotopic pattern and determination of UV max (nm) – through UV-PDA studies. Adulteration was then detected in AHPs primarily through comparison of EICs at accurate m/z for molecular ion peaks and R_T matching with the standard. It was confirmed by spiking with the standards, and matching mass spectrum, accurate mass, R_T of qualifier fragments, isotopic pattern and UV spectrum of the standards with the adulterant peaks in AHPs. Dexamethasone and diclofenac were detected as adulterants in ten AHPs whereas one AHP tested positive for piroxicam and another for dexamethasone. All the adulterated products were sold by the healthcare practitioners, while no product marketed by manufacturers or chemist shops had this problem. The study showed that LC-MS/TOF-based screening could be used as a rapid approach to monitor adulteration of steroids and anti-inflammatory drugs in AHPs.

Keywords: adulteration; Ayurvedic/herbal healthcare products; LC-MS/TOF; non-steroidal anti-inflammatory drugs steroids

Introduction

Ayurvedic/herbal healthcare products (AHPs) are used worldwide as effective and relatively safe alternatives to allopathic drugs. However, AHPs are not regulated as strictly as modern medicines, so there is no systematic control over their quality.[1,2] In many cases they are sold by practitioners of alternative medicines and prescribed for the treatment of chronic ailments. To prove that their products act effectively, the practitioners unethically add synthetic drugs to AHPs. This is a serious safety concern as long-term use of some of the synthetic drugs without a physician's intervention could have adverse effects.[3,4]

Reports of adulteration of AHPs have appeared from time to time. The drugs used for adulteration include (1) steroids and non-steroidal anti-inflammatory drugs (NSAIDs) for the treatment of rheumatoid arthritis, allergy and asthma;[1,2,4–29] (2) PDE-5 inhibitors for sexual dysfunction;[27,30–35] (3) sibutramine as anorectic;[36–38] and (4) sulfonylureas (chlorpropamide, tolbutamide and glibenclamide) in antidiabetic formulations.[4,39] Among these, the most prevalent is the adulteration of AHPs with synthetic steroids and NSAIDs.[2,4,27] The most commonly found steroidal adulterants include dexamethasone, hydrocortisone, prednisolone, betamethasone, cortisone and triamcinolone and the NSAIDs used include diclofenac, mefenamic acid, indomethacin, paracetamol, phenacetin and phenylbutazone.[1,4–6,27] The steroids are associated with various detrimental effects, including muscle weakness, osteoporosis, cataracts, glaucoma and iatrogenic Cushing syndrome.[40] On the other hand, adverse reactions such as dyspepsia, nausea, vomiting, gastric damage with risk of haemorrhage, skin reactions, reversible renal insufficiency, nephropathy (mainly with paracetamol) and bone marrow depression are the risk factors associated with the use of NSAIDs.[41,42]

Adulteration of AHPs with steroids and NSAIDs has been reported mostly from China, Singapore, Pakistan, South Africa and India.[2,3,5,7–10,12,14–16,18,19,23,25–29] The methods used for detection of adulteration have been based on medical symptoms or analytical approaches such as UV colorimetry, TLC, HPLC and LC-MS. Adulteration of animal food products and environmental water samples with steroids and NSAIDs has been reviewed.[43–48]

The purpose of the present study was to propose a simple and high-throughput strategy using LC-MS/TOF for unequivocal detection and characterization of adulteration of AHPs with steroids and NSAIDs. The reason for the selection of LC-MS/TOF for the purpose was its inherent advantages, including wide dynamic range, simultaneous acquisition of fragmentation and accurate mass data, and ability to generate precise isotopic patterns.[49] The strategy developed was applied to check adulteration in 58 AHPs procured from different parts of India.

* *Correspondence to: Saranjit Singh, NIPER, Pharm. Analysis, Sector-67, SAS Nagar, Mohali, India 160 062. E-mail: ssingh@niper.ac.in*

Department of Pharmaceutical Analysis, National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, S.A.S. Nagar, Mohali 160 062, Punjab, India

Experimental

Materials

Standards of steroids (triamcinolone, prednisone, cortisone, betamethasone, prednisolone, hydrocortisone, dexamethasone, methylprednisolone and fludrocortisone) and NSAIDs (paracetamol, aspirin, phenacetin, rofecoxib, piroxicam, ketorelac, ibuprofen, meloxicam, nimesulide, ketoprofen, naproxen, aceclofenac, celecoxib, diclofenac, mefenamic acid and indomethacin) were procured from manufacturers of different active pharmaceutical ingredients (API) and formulations. Of the total 58 AHPs (AHP 1–AHP 58), 15 were purchased from chemist shops in the vicinity (AHP 1–15), while the remaining ones were procured from chemist shops (AHP 16–45) and practitioners of alternative medicines (AHP 46–58) from other states of India (14 from Gujarat, seven from Andhra Pradesh, five from Himachal Pradesh, five from Haryana, five from Maharashtra, four from Madhya Pradesh and three from Delhi). HPLC-grade methanol was procured from J. T. Baker (Phillipsburg, NJ, USA). Buffer salts and all other chemicals were of analytical reagent grade. Ultra pure water obtained from an ELGA (Wycombe, Bucks, England) water purification unit was used throughout the studies.

Instruments

The LC-MS/TOF studies were carried out with a system in which the LC part consisted of 1100 series HPLC (Agilent Technologies, Waldbronn, Germany) and the MS part comprised of a MicrOTOF-Q spectrometer (Bruker Daltonics, Bremen, Germany). The components of LC were an on-line degasser (G1379A), a binary pump (G131A), an auto-injector (G1313A), a column oven (G1316A) and a diode-array detector (G1315B). The system was controlled by Hyphenation Star (version 3.1) and MicrOTOF Control (version 2.0) software. Chromatographic separations were achieved on a C18 column (250 mm × 4.6 mm i.d., particle size 5 µm) procured from Princeton Chromatography Inc. (Cranbury, NJ, USA).

Sample preparation

A common sample preparation method was used whereby APIs were dissolved in methanol : water (80 : 20). The marketed formulations in the form of capsules, tablets or powders were extracted using the same solvent and sonicated for 40 min. The final concentration for each standard was kept between 200–400 µg/ml as per their response in HPLC. In case of herbal samples, 500 mg powder was taken in each case, and was diluted with 10 ml of the solvent and sonicated. The extract was then centrifuged at 10 000 rpm for 20 min at 25 °C and the supernatant was subjected to HPLC and LC-MS/TOF analyses.

HPLC method development and validation

The target during HPLC method development was to adjust resolution of all the 25 standard drugs in a single run, irrespective of consideration of peak width and total run time. This was to simplify the whole process of the detection of adulteration. For this, the following gradient was employed: T_{min}/methanol:0.01 M ammonium acetate (v/v); $T_{0.01-10}$/45 : 55 (v/v), T_{15}/50 : 50 (v/v), T_{25}/55 : 45 (v/v), T_{30-45}/56 : 44 (v/v), T_{50}/80 : 20 (v/v) and T_{55-63}/45 : 55 (v/v). UV spectra were extracted at 242 nm for steroids, and 230 nm and 330 nm for NSAIDs. The injection volume and flow rate were 10 µl and 1 ml/min, respectively.

Table 1. Parameters of the MS/TOF method developed in this study

MS/TOF Parameter		**Mode** *APCI +ve*	*ESI −ve*
Source	End plate offset (V)	−500	−500
	Capillary (V)	−4500	+4500
	Nebuliser (Bar)	1.2	0.5
	Dry gas (L/min)	4	5
	Dry temperature (°C)	200	180
	Vaporizer temperature (°C)	375	–
Transfer	Funnel 1 RF (Vpp)	150.0	50.0
	Funnel 2 RF (Vpp)	400.0	100.0
	ISCID energy (eV)	0	0
	Hexapole RF (Vpp)	400.0	140.0
Quadrupole	Ion energy	5.0	5.0
	Low mass (m/z)	300.00	150.0
Collision cell	Collision energy (eV/z)	10.0	10.0
	Transfer time (µs)	150.0	100.0
	Collision RF (Vpp)	300.0	200.0
	Prepulse storage (µs)	3.0	4.0
Detector	Source (V)	−1200	−1200
Mass range	50–600 (amu)		
Scan rate	3 (Hz)		

The HPLC method developed was validated with respect to linearity, intra-day, inter-day and intermediate precision and accuracy. To establish linearity and range, solutions of dexamethasone, diclofenac and piroxicam were prepared in the range of 1–35, 50–400 and 50–500 µg/ml, respectively, followed by their analysis in triplicate by the developed HPLC method. Intra- and inter-day precision were investigated by analysing 15, 20 and 25 µg/ml of dexamethasone, 200, 250 and 300 µg/ml of diclofenac and 100, 200 and 300 µg/ml of piroxicam three times on the same day and on three consecutive days, respectively. To determine intermediate precision, the brand of the column was changed and the whole experiment was conducted on a different instrument. Accuracy was determined by spiking AHP extract with the above three concentrations of dexamethasone, diclofenac and piroxicam, in triplicate. For the determination of limits of detection (LOD) and quantification (LOQ), the samples showing no signal at R_T of the standard were considered as blank. The concentration in the spiked samples that gave 3.3 and 10 times higher response than noise in the blank were taken as LOD and LOQ, respectively.[50] The same procedure was used to establish LOD for adulterants in the EIC method. The amounts of synthetic adulterants in AHP samples were determined using the linear regression equations.

Optimization of LC-MS/TOF parameters and instrument calibration

The instrument parameters were optimized for both the APCI and ESI modes to achieve optimum fragmentation of all the drugs. Acceptable fragmentation for the steroidal and non-steroidal drugs, except aspirin and ibuprofen, was observed in positive APCI mode. Negative ESI mode provided optimum fragmentation of aspirin and ibuprofen. The employed instrument parameters are provided in Table 1.

The LC-MS/TOF was calibrated in APCI and ESI modes using tuning mix solutions supplied by the vendor (Agilent Technologies). Both the solutions were diluted to a suitable concentration using

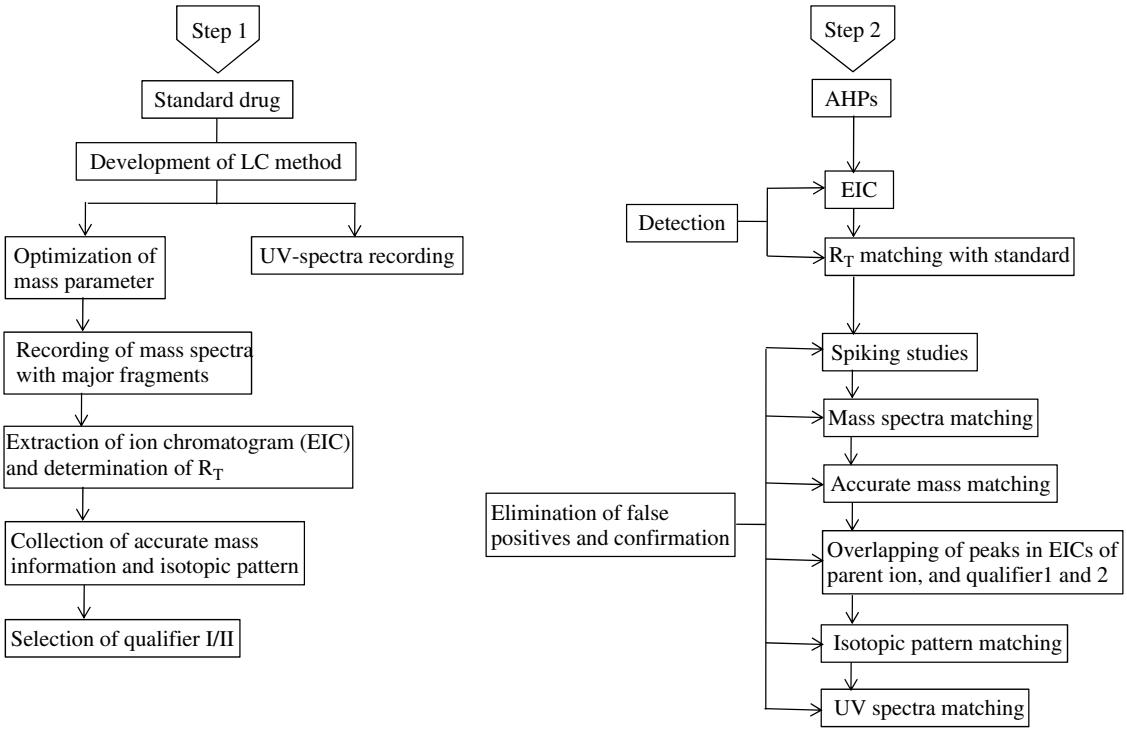

Figure 1. Generic strategy for screening of steroids and NSAIDs in AHPs.

a mixture of ACN-water (95 : 5% v/v). The masses in positive APCI mode were corrected by internal reference ions of m/z 322.0481 ($C_6H_{19}O_6N_3P_3$), 622.0290 ($C_{12}H_{19}O_6N_3P_3F_{12}$) and 922.0098 ($C_{18}H_{19}O_6N_3P_3F_{24}$), whereas those of negative ESI mode were corrected by internal reference ions of m/z 431.9823 (M (601)-R), 601.9790 ($C_{12}HON_3F_{21}$) and 1033.9756 ($C_{18}H_{18}O_6N_3P_3F_{24}$).

Development of LC-MS/TOF strategy for the detection and confirmation of adulteration

A generalized LC-MS/TOF-based strategy (Figure 1) was developed for the detection and characterization of synthetic adulterants in AHPs. It involved two steps: collecting relevant mass-related information on standard drugs and the application of this to check adulteration in AHP samples. Characteristic LC-MS/TOF information, like mass spectrum, molecular ion peaks, accurate masses, two qualifier major fragments, EIC spectrum, R_T, and isotopic patterns of molecular ion peaks, was collected for all the drug standards. UV spectra were also recorded from LC-PDA studies between 200–400 nm. For checking the adulteration, all the herbal formulations were initially screened by utilizing the m/z values of molecular ion peaks of standard drugs to acquire EICs. The R_T values of the adulterants in the AHPs were subsequently matched with those of the standards. The detected adulterants were then confirmed by spiking with the standards and matching of (1) full mass spectrum, (2) accurate mass values, (3) EIC peak of the parent ions with two qualifier major fragments, (4) isotopic pattern, and (5) UV spectrum.

Results and Discussion

Development of the HPLC method

The developed gradient HPLC method was able to give peaks of all the investigated drug standards within 60 min. The method could even be extended to the LC-MS system, as evidenced by EICs in Figure 3. The broadening and tailing of some of the drug peaks was ignored as the initial primary objective was to detect adulteration only. The method was subsequently validated for quantitation of drugs detected as adulterants in AHPs, as discussed below.

Collection of characteristic information on synthetic adulterants

Mass spectra were first recorded for all the standards to determine accurate molecular mass and fragmentation/isotopic patterns. In each case, two major fragment peaks were identified, which were considered as qualifier peaks 1 and 2. The mass spectra for each standard, with qualifier peaks labelled as Q_1 and Q_2, are shown in Figure 2. The m/z values for the parent peaks were subsequently used to record EICs for each standard. They are shown in Figure 3.

Information such as mass mode, R_T, accurate mass, qualifier fragment mass, isotopic pattern of molecular ion peaks and UV_{max}, is presented for the steroids and NSAIDs in Tables 2 and 3, respectively.

Detection and confirmation of adulteration in AHPs

The results of EIC-based screening of all the AHPs highlighted that AHP 46 to AHP 55 had characteristic peaks at R_T 32.9 min and 54.8 min, identical to the resolution of peaks for dexamethasone and diclofenac, respectively. Similarly, AHP 57 and AHP 58 showed characteristic peaks in EICs akin to piroxicam and dexamethasone, respectively. These suspect samples were spiked with standards of the detected adulterants. Spiked chromatograms in Figure 4, a and a′ depict increased intensity of peaks at R_T 32.9 min and 54.8 min in AHP 46 after spiking with standard dexamethasone and diclofenac, respectively. Likewise, there was an increase in intensity of the peak at R_T 19.6 min in AHP 57 (Figure 4b

and b′) and the peak at R_T 32.9 min in AHP 58 (Figure 4c and c′) after spiking with standards of piroxicam and dexamethasone, respectively.

The mass spectra of the detected adulterants at R_T 32.9 min and 54.8 min in AHP 46 to AHP 55 also matched those of the standard dexamethasone and diclofenac, respectively (Figure 5a and a′). Similarly, the mass spectra of the detected adulterants at R_T 19.6 min in AHP 57 (Fig. 5b and b′) and at R_T 32.9 min in AHP 58 (Fig. 5c and c′) were the same as those of piroxicam and dexamethasone, respectively.

Accurate mass studies were used to eliminate false positive results, because many structures could show a single nominal mass but all of them would differ in their accurate masses. So, the accurate mass of the peaks for molecular ions, qualifier 1 and qualifier 2 of the detected synthetic adulterants in

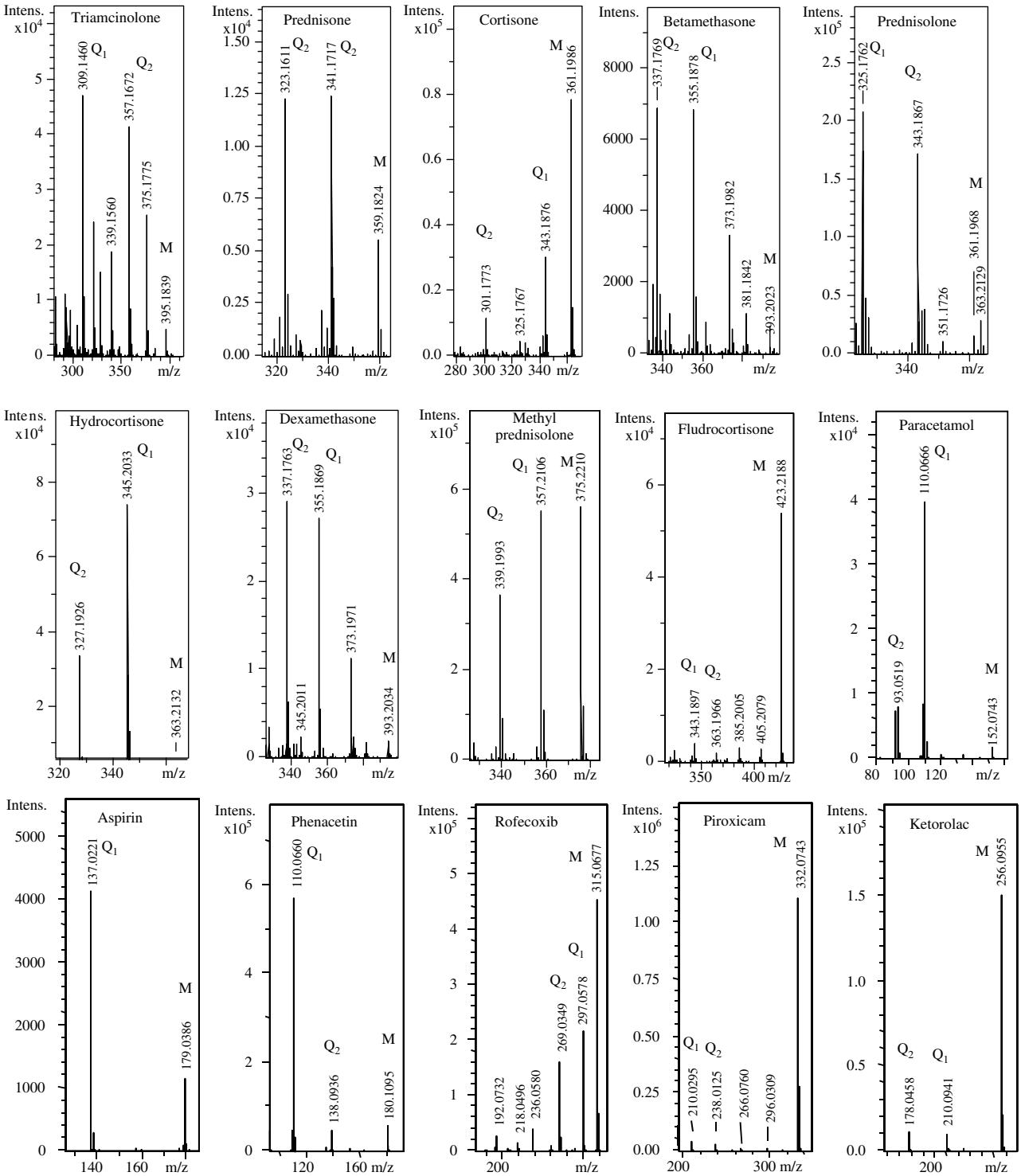

Figure 2a. Mass spectra of steroids and NSAIDs (M: Molecular ion peak, Q_1: Qualifier 1, Q_2: Qualifier 2).

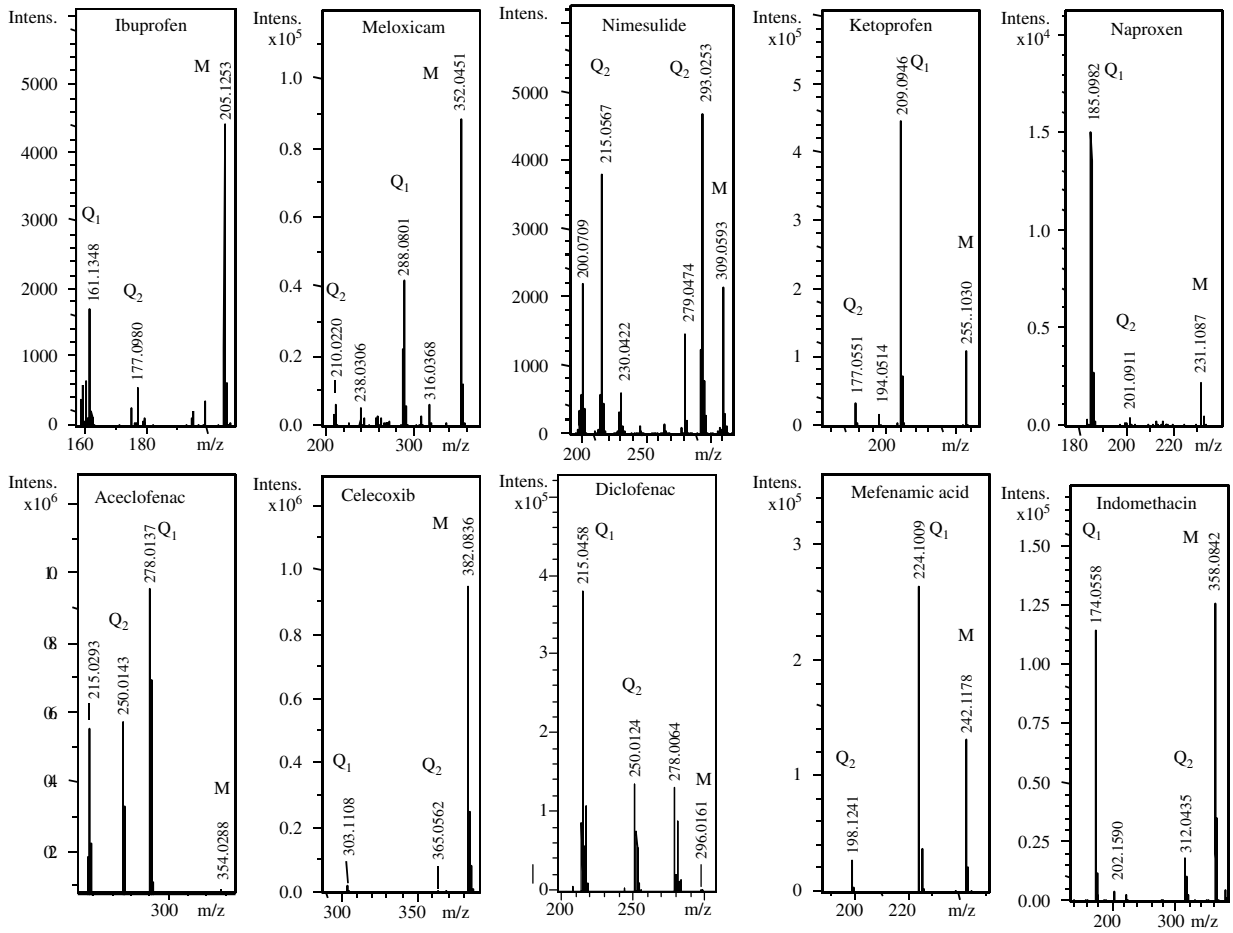

Figure 2b. (*Continued*).

Table 2. LC-MS/TOF data for steroids

Drug	Mode	R_T	Accurate mass	Qualifier 1	Qualifier 2	Isotopic pattern	λ max (nm)
Triamcinolone	APCI +ve	13.1	395.1839	309.1460	357.1672	100 : 23 : 3	239
Prednisone	APCI +ve	20.0	359.1824	341.1717	323.1611	100 : 23 : 3	239
Cortisone	APCI +ve	21.2	361.1986	343.1876	301.1773	100 : 23 : 3	328
Betamethasone	APCI +ve	22.5	393.2023	355.1878	337.1769	100 : 24 : 3	238
Prednisolone	APCI +ve	24.7	361.1968	325.1763	343.1867	100 : 23 : 3	242
Hydrocortisone	APCI +ve	32.0	363.2132	345.2033	327.1926	100 : 23 : 3	242
Dexamethasone	APCI +ve	32.9	393.2034	355.1869	337.1763	100 : 24 : 3	238
Methylprednisolone	APCI +ve	34.4	375.2210	357.2106	339.1993	100 : 24 : 3	243
Fludrocortisone	APCI +ve	34.4	423.2188	343.1897	363.1966	100 : 25 : 3	239

AHPs, were matched with that of the standards. In case of the detected adulterant at R_T 32.9 min, accurate masses were similar to the theoretical mass for dexamethasone. Likewise, in the case of the detected adulterant at R_T 54.8 min in AHP 46 to AHP 55, the accurate masses showed presence of diclofenac, and in AHP 57, the accurate masses of the peak at R_T 19.6 min matched with piroxicam. Similarly, in AHP 58, the accurate masses matched with dexamethasone (Figure 5). Moreover, R_T values of molecular ion peak, and peaks for qualifier 1 and qualifier 2 were strongly superimposed in each case, thus verifying the adulteration. Similar superimposing R_T values in EICs were observed even for the remaining samples containing synthetic adulterants (AHP 47–55, AHP 58). The isotopic patterns of the molecular ion peaks and UV spectra were similar for the adulterant in test samples and the drug standards.

Validation of the method and quantitative estimation of dexamethasone, diclofenac and piroxicam

The three drugs that were detected as adulterants in AHPs i.e., dexamethasone, diclofenac and piroxicam – resolved with reasonably well-shaped peaks on an LC column, as shown in Figure 3, validating the HPLC method for quantitative

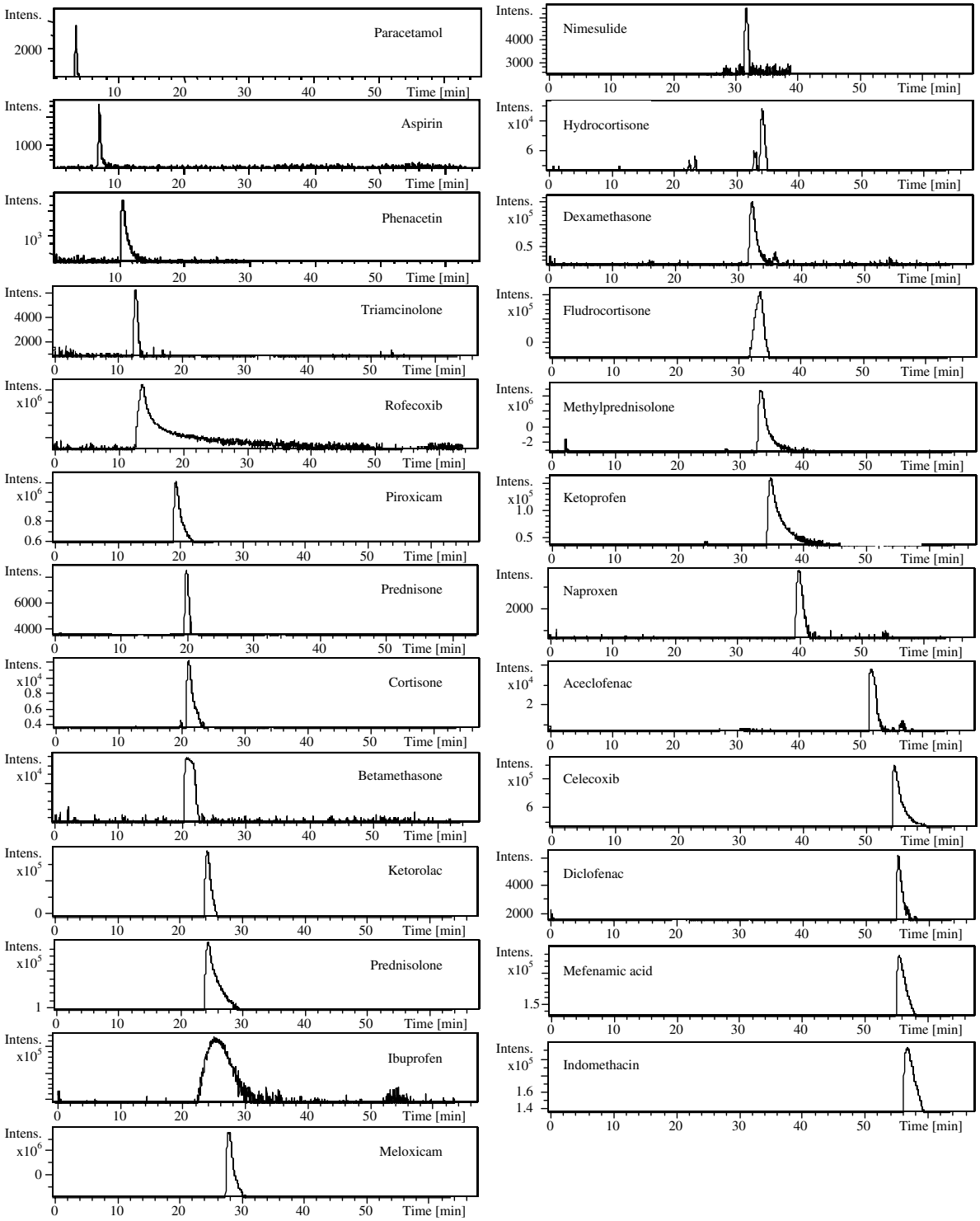

Figure 3. Extracted ion chromatograms of steroids and NSAIDs.

determination of these three drugs. The ranges were selected based on relative human doses of the three drugs. The linearity data for dexamethasone, diclofenac and piroxicam in the concentration range of 1 to 35 µg/ml, 50 to 400 µg/ml and 50 to 500 µg/ml, respectively, are given in Table 4. The percentage RSD for each concentration was less than 0.5%. The same for intra- and inter-day precision studies at three different concentrations was also less than 1.5% (Table 5). Similar resolution behaviour was observed by repeating the experiment on two different HPLC systems by two different analysts. Good recoveries were also obtained when herbal samples were spiked with 15, 20 and 25 µg/ml of dexamethasone, 200, 250 and 300 µg/ml of diclofenac and 100, 200 and 300 µg/ml of piroxicam (Table 6). The respective LOD and LOQ values of the HPLC method were 0.29 and 0.97 µg/ml for dexamethasone, 0.4 and 1.32 µg/ml for diclofenac and 10.4 and 34.32 µg/ml for piroxicam. The LOD

Table 3. LC-MS/TOF data of NSAIDs

Drug	Mode	R_T	Accurate mass	Qualifier 1	Qualifier 2	Isotopic pattern	λ max (nm)
Paracetamol	APCI +ve	3.4	152.0743	110.0666	93.0519	100 : 9	248
Aspirin	ESI −ve	6.9	179.0386	137.0221	–	100 : 8	225, 296
Phenacetin	APCI +ve	10.6	180.1095	110.0660	138.0936	100.10	248
Rofecoxib	APCI +ve	13.9	315.0677	297.0578	269.0349	100 : 18 : 5	285
Piroxicam	APCI +ve	19.6	332.0743	210.0295	238.0125	100 : 16 : 5	334
Ketorolac	APCI +ve	24.5	256.0955	210.0941	178.0458	100 : 16 : 1	254
Ibuprofen	ESI −ve	25.1	205.1253	161.1348	177.0980	100 : 14	226
Meloxicam	APCI +ve	28.1	352.0451	288.0801	210.0220	100 : 15 : 9	355
Nimesulide	APCI +ve	31.7	309.0593	293.0474	215.0567	100 : 14 : 5	350
Ketoprofen	APCI +ve	34.7	255.1030	209.0946	177.0551	100 : 17 : 1	260
Naproxen	APCI +ve	39.7	231.1087	185.0982	201.0911	100 : 15 : 1	229
Aceclofenac	APCI +ve	51.4	354.0288	278.0137	250.0143	100 : 17 : 64	274
Celecoxib	APCI +ve	54.1	382.0836	303.1108	365.0562	100 : 18 : 5	252
Diclofenac	APCI +ve	54.8	296.0161	215.0458	250.0124	100 : 15 : 64	282
Mefenamic acid	APCI +ve	55.2	242.1178	224.1009	198.1241	100 : 16 : 1	285, 340
Indomethacin	APCI +ve	56.4	358.0842	174.0558	312.0435	100 : 19 : 32	318

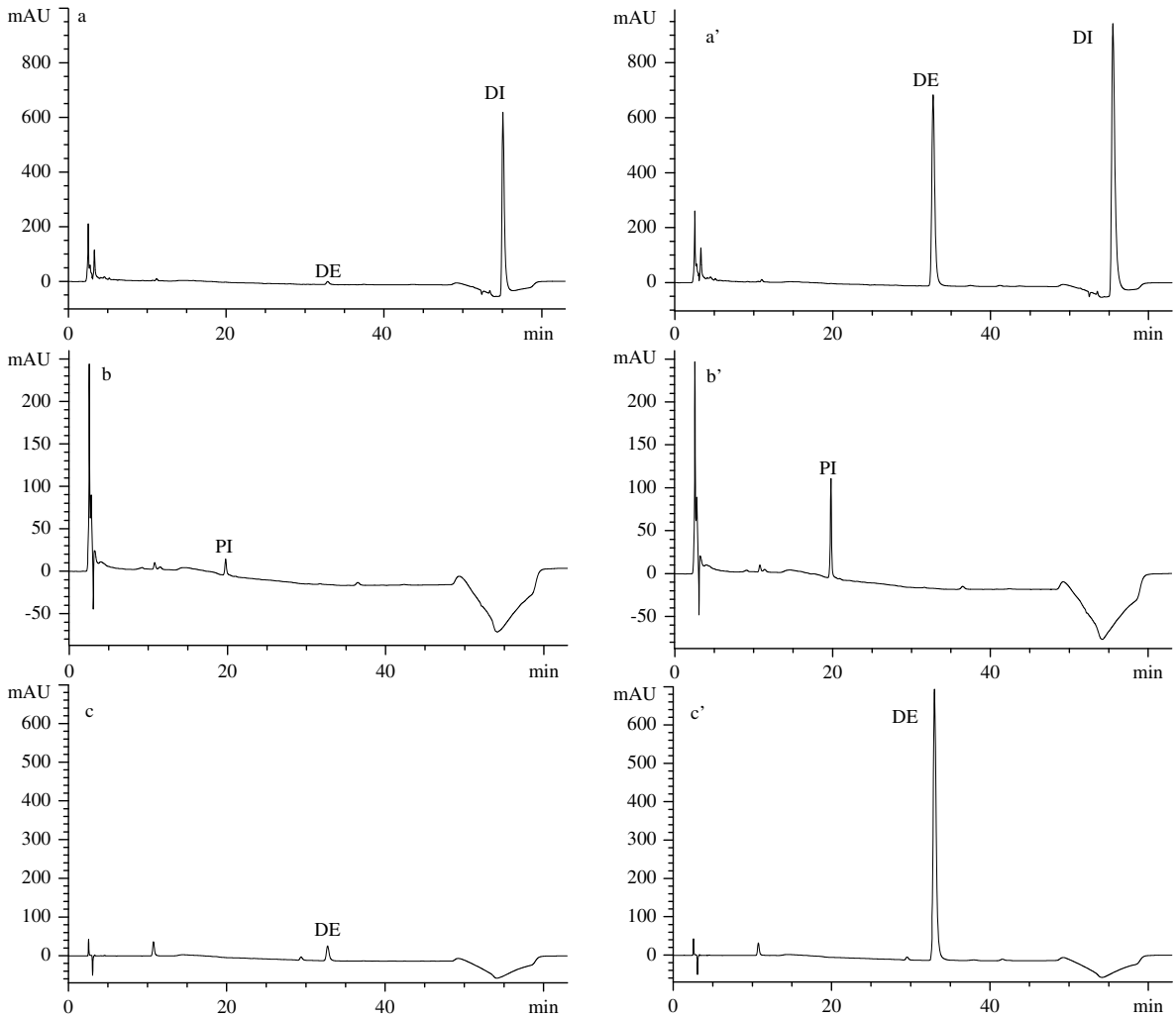

Figure 4. Chromatograms of (a) AHP 46, (a′) dexamethasone (DE) and diclofenac (DI) spiked in AHP 46, (b) AHP 57, (b′) piroxicam (PI) spiked in AHP 57, (c) AHP 58, (c′) dexamethasone spiked in AHP 58.

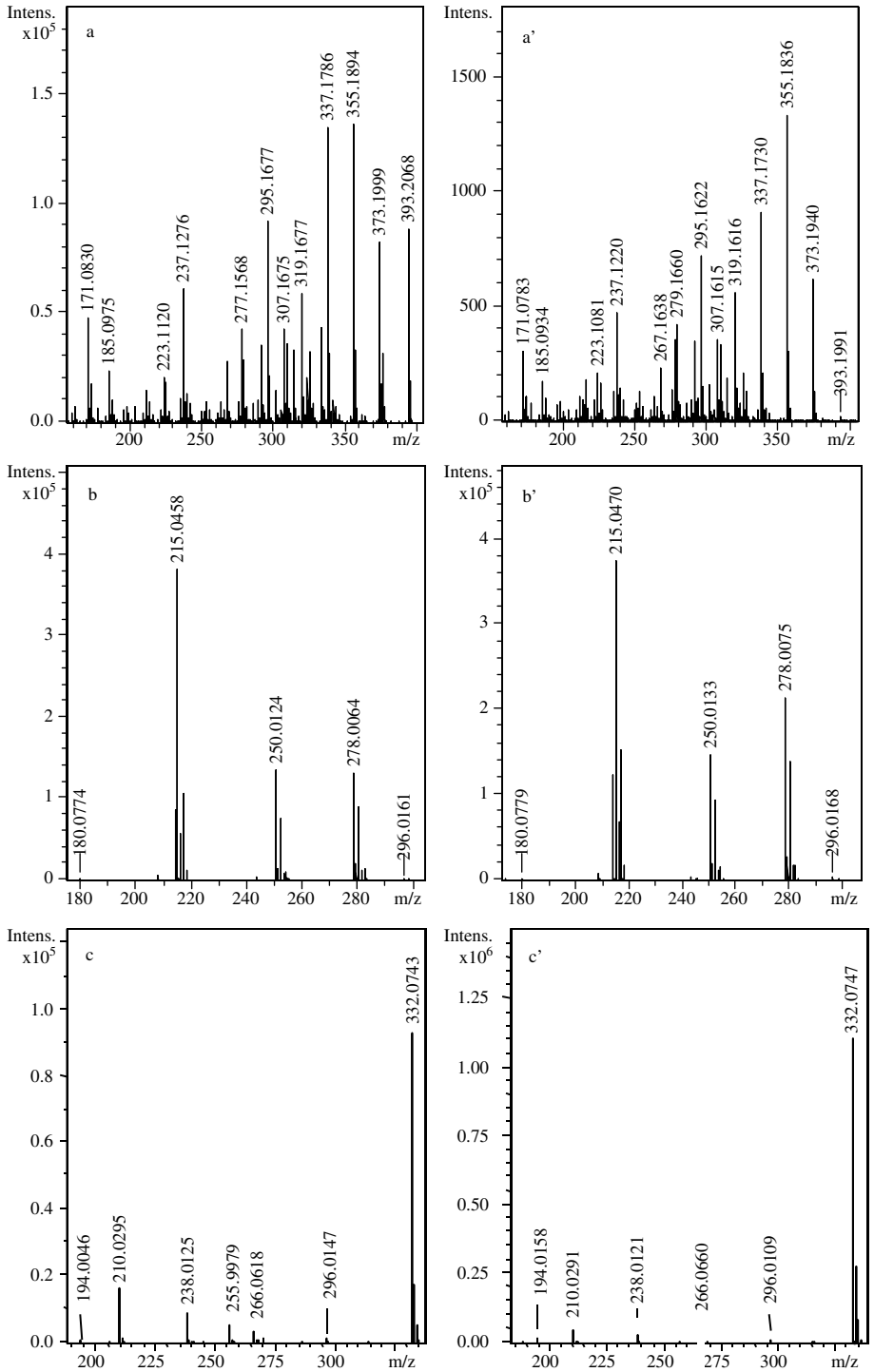

Figure 5. Mass spectra of (a) dexamethasone, (a′) detected adulterant at R_T 32.9 min in AHP 46, (b) diclofenac, (b′) detected adulterant at R_T 54.8 min in AHP 46, (c) piroxicam and (c′) detected adulterant at R_T 19.6 min in AHP 57.

Table 4. Linearity data of drug used for adulteration (n = 3)

Drug	Concentration range (µg/ml)	Equation for regression line	R^2 value
Dexamethasone	1–35	$y = 23.37x + 15.55$	0.998
Diclofenac	50–400	$y = 10.83x + 386.7$	0.997
Piroxicam	50–500	$y = 1.227x - 1.100$	0.997

values of dexamethasone, diclofenac and piroxicam determined for the EIC method were 35, 55 and 250 ng/ml, respectively.

Based on the calibration equations of the HPLC method, the amounts of dexamethasone, diclofenac and piroxicam were determined per dose of the adulterated AHPs. As seen from the data in Table 7, the amount of adulteration in sample was less than the usual dose of dexamethasone (0.5–1.0 mg); diclofenac (70–150 mg) and piroxicam (20–50 mg).

Table 5. Intra-day and inter-day precision studies (n = 3)

Drug	Added concentration (μg/ml)	Measured concentration ± S.D.(μg/ml), R.S.D. (%)	
		Intra-day precision	*Inter-day precision*
Dexamethasone	15	15.26 ± 2.05, 0.55	15.33 ± 5.35, 1.43
	20	20.37 ± 1.12, 0.23	20.29 ± 5.40, 1.10
	25	25.49 ± 1.12, 0.22	25.63 ± 5.36, 0.87
Diclofenac	200	200.72 ± 4.96, 0.19	200.73 ± 9.95, 0.39
	250	250.93 ± 1.20, 0.04	250.41 ± 10.51, 0.34
	300	300.52 ± 2.42, 0.07	299.98 ± 4.60, 0.13
Piroxicam	100	101.35 ± 1.01, 0.82	101.63 ± 1.41, 1.14
	200	200.84 ± 2.42, 0.99	199.21 ± 1.50, 0.62
	300	300.81 ± 2.65, 0.72	300.63 ± 1.00, 0.27

Table 6. Recovery studies

Drug	Spiked concentration (μg/ml)	Calculated spiked concentration (μg/ml) ± S.D., R.S.D. (%)	Recovery (%)
Dexamethasone	15	15.21 ± 3.30, 0.89	101.42
	20	20.43 ± 4.24, 0.86	102.19
	25	25.61 ± 1.08, 0.18	102.45
Diclofenac	200	199.22 ± 8.87, 0.35	99.61
	250	253.79 ± 27.67, 0.88	101.52
	300	298.44 ± 8.03, 0.22	99.48
Piroxicam	100	102.47 ± 1.01, 0.81	102.47
	200	198.66 ± 0.49, 0.20	99.33
	300	302.71 ± 0.58, 0.16	100.90

Table 7. Amount of synthetic adulterants per dose of AHPs

AHPs	Dose of AHPs (mg)	Dexamethasone (mg)	Diclofenac (mg)	Piroxicam (mg)
AHP 46	500	0.20	14.87	–
AHP 47	500	0.04	9.82	–
AHP 48	500	0.16	9.80	–
AHP 49	500	0.19	13.92	–
AHP 50	500	0.20	11.66	–
AHP 51	500	0.17	6.49	–
AHP 52	500	0.18	7.26	–
AHP 53	500	0.18	6.58	–
AHP 54	500	0.18	7.90	–
AHP 55	500	0.23	10.77	–
AHP 57	50	–	–	0.37
AHP 58	100	0.20	–	–

Conclusion

A novel and simple LC-MS/TOF-based scientific strategy was developed to detect adulteration of AHPs with steroids and NSAIDs. The strategy was applied to 58 AHPs collected from various parts of India. Ten AHPs were found to contain dexamethasone and diclofenac, one contained piroxicam and another dexamethasone as synthetic adulterants. All the AHPs with adulteration were those dispensed by practioners of alternative medicines. All products marketed by other means were free of the problem. The continued indiscriminate use of steroids and/or NSAIDs in AHPs by practioners in India is a matter of concern as unsuspecting patients may be exposed to side effects of long-term exposure to synthetic drugs taken without medical supervision.

References

[1] E. Ernst, *Pharm. J.* **2005**, *275*, 167.
[2] S. K. Gupta, T. Kaleekal, S. Joshi, *Pharmacoepidemiol. Drug Saf.* **2000**, *9*, 599.
[3] J. I. Boullata, A. M. Nace, *Pharmacother.* **2000**, *20*, 1.
[4] E. Ernst, *Pharmacoepidemiol. Drug Saf.* **2004**, *13*, 767.
[5] S. Ahmed, M. Riaz, *Chromatographia* **1991**, *31*, 67.
[6] J. K. Dowman, F. H. Khattak, S. Elliott, T. M. T. Sheehan, K. A. Grindulis, *Rheumatology* **2006**, *45*, 1309.
[7] T. Snyman, M. J. Stewart, A. Grove, V. Steenkamp, *Ther. Drug Monit.* **2005**, *27*, 86.
[8] R. J. Ko, *N. Engl. J. Med.* **1998**, *339*, 847.
[9] C. A. Ries, M. A. Sahud, *JAMA* **1975**, *231*, 352.
[10] P. M. Brooks, R. M. Lowenthal, *Med. J. Aust.* **1977**, *2*, 860.
[11] P. G. J. Forster, M. Calverley, S. Hubball, B. McConkey, *BMJ* **1979**, *2*, 308.
[12] L. Offerhaus, M. N. G. Dukes, *BMJ* **1979**, *2*, 668.
[13] R. W. Bury, R. O. Fullinfaw, D. Barraclough, K. D. Muirden, R. F. W. Moulds, T. Anghie, *Med. J. Aust.* **1987**, *146*, 324.
[14] A. M. Joseph, T. Biggs, M. Garr, J. Singh, F. A. Lederle, *N. Engl. J. Med.* **1991**, *3*, 62.
[15] J. O. Driscoll, A. D. Burden, T. P. Kingston, *Br. J. Dermatol.* **1992**, *127*, 543.
[16] J. R. Hughes, E. M. Higgins, A. C. Pembroke, *Br. J. Dermatol.* **1994**, *130*, 261.
[17] B. I. V. Stricht, O. E. Parvais, R. J. Vanhaelen-Fastré, M. H. Vanhaelen, *BMJ* **1994**, *308*, 1162.
[18] R. A. C. Graham-Brown, J. F. Bourke, G. Bumphrey, *BMJ* **1994**, *308*, 473.
[19] B. Wood, J. Wishart, *N.Z. Med. J.* **1997**, *110*, 420.
[20] E. Gertner, P. S. Marshall, D. Filandrinos, A. S. Potek, T. M. Smith, *Arthritis Rheum.* **1995**, *38*, 614.
[21] J. A. Goldman, G. Myerson, *Arthritis Rheum.* **1991**, *34*, 1207.
[22] H. Hatanaku, *Public Health Inst. Bull.* **1984**, *19*, 106.
[23] W. F. Huang, K. C. Wen, M. L. Hsiao, *J. Clin. Pharmacol.* **1997**, 344.
[24] F. M. Keane, S. E. Munn, A. W. P. Vivier, N. F. Taylor, E. M. Higgins, *BMJ* **1999**, *318*, 563.
[25] A. B. Abt, J. Y. Oh, R. A. Huntington, K. K. Burkhart, *Arch. Intern. Med.* **1995**, *155*, 211.
[26] H. L. Koh, S. O. Woo, *Drug Saf.* **2000**, *23*, 351.
[27] E. Ernst, *J.Inter. Med.* **2002**, *252*, 107.
[28] R. Krishnaprasad, V. V. Pillay, R. R. Rajesh, N. Vishnupriya, *J. Indian Soc. Toxicol.* **2005**, *1*, 20.
[29] Y. F. Chen, H. M. Chern, M. H. Chou, M. F. Ling, *Ann. Emerg. Med.* **1995**, *26*, 717.

[30] S. Singh, B. Prasad, A. A. Savaliya, R. P. Shah, V. M. Gohil, A. Kaur, *Trends Anal. Chem.* **2009**, *28*, 13.
[31] J. C. Reepmeyer, J. T. Woodruff, D. A. Avingnon, *J. Pharm. Biomed. Anal.* **2007**, *43*, 1615.
[32] M. J. Bogusz, H. Hassan, E. Al-Enazi, Z. Ibrahim, M. Al-Tufail, *J. Pharm. Biomed. Anal.* **2006**, *41*, 554.
[33] Q. Liang, J. Qu, G. Luo, Y. Wang, *J. Pharm. Biomed. Anal.* **2006**, *40*, 305.
[34] S. R. Gratz, C. L. Flurer, K. A. Wolnik, *J. Pharm. Biomed. Anal.* **2004**, *36*, 525.
[35] P. Zou, S. S. Y. Oh, P. Hou, M. Y. Low, H. L. Koh, *J. Chromatogr. A* **2006**, *1104*, 113.
[36] Y. P. Yuen, C. K. Lai, W. T. Poon, S. W. Ng, A. Y. W. Chan, T. W. L. Mak, *Hong Kong Med. J.* **2007**, *13*, 216.
[37] J. Jung, M. Hermanns-Clausen, W. Weinmann, *Forensic Sci. Int.* **2006**, *161*, 221.
[38] P. Zou, S. S. Y. Oh, K. H. Kiang, M. Y. Low, B. C. Bloodworth, *Rapid Commun. Mass Spectrom.* **2007**, *21*, 614.
[39] Y. Ku, L. Chag, L. Ho, J. Lin, *J. Pharm. Biomed. Anal.* **2003**, *33*, 329.
[40] H. P. Rang, M. M. Dale, J. M. Ritter, P. K. Moore, *Pharmacology*, 5 edn, Elsevier: New Delhi, India, **2003;**, pp. 412–419.
[41] H. P. Rang, M. M. Dale, J. M. Ritter, P. K. Moore, *Pharmacology*, 5 edn, Elsevier: New Delhi, India, **2003;**, pp. 244–252.
[42] D. V. Derle, K. N. Gujar, B. Sagar, *Indian J. Pharm. Sci.* **2006**, *68*, 409.
[43] X. Cui, B. Shao, R. Zhao, Y. Yang, J. Hu, X. Tu, *Rapid Commun. Mass Spectrom.* **2006**, *20*, 2355.
[44] A. Gentili, *Trends Anal. Chem.* **2007**, *26*, 595.
[45] A. Gentili, *Anal. Bioanal. Chem.* **2007**, *387*, 1185.
[46] M. Gros, M. Petrović, D. Barceló, *Anal. Bioanal. Chem.* **2006**, *386*, 941.
[47] M. Petrovic, M. D. Hernando, M. S. Dıaz-Cruz, D. Barcelo, *J. Chromatogr. A* **2005**, *1067*, 1.
[48] A. A. M. Stolker, U. A. T. Brinkman, *J. Chromatogr. A* **2005**, *1067*, 15.
[49] I. V. Chernushevich, A. V. Loboda, B. A. Thomson, *J. Mass Spectrom.* **2001**, *36*, 849.
[50] ICH, Q2(R1), in: *International Conference on Harmonisation*, IFPMA, Geneva, **2005**.