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High-performance liquid chromatography analysis of anti-inflammatory pharmaceuticals with ultraviolet and electrospray-mass spectrometry detection in suspected counterfeit homeopathic medicinal products

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Abstract

A simple high-performance liquid chromatography (HPLC) method with both ultraviolet (UV) and electrospray ionisation mass spectrometry (ESI-MS) detection has been developed for the determination of seven pharmaceuticals in counterfeit homeopathic preparations. Naproxen, Ketoprofen, Ibuprofen, Diclofenac, Piroxicam, Nimesulide and Paracetamol were separated by reversed phase chromatography with acetonitrile–water (0.1% acetic acid) mobile phase, and detected by UV at 245 nm and by ESI-MS in negative ionisation mode with the exception of Paracetamol which was detected in positive ionisation mode. Benzoic acid was used as internal standard (IS). This method was successfully applied to the analysis of homeopathic preparations like mother tinctures, solutions, tablets, granules, creams, and suppositories. Linearity was studied with UV detection in the 50–400 $\mu g\,mL^{-1}$ range and with ESI-MS in the 0.1–50 $\mu g\,mL^{-1}$ range. Good correlation coefficients were found in both UV and ESI-MS. Detection limits ranged from 0.18 to 41.5 ng in UV and from 0.035 to 1.00 ng in ESI-MS.
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1. Introduction

A counterfeit pharmaceutical product is defined as a product that is mislabelled deliberately and fraudulently with respect to its identity or source. Counterfeit products may include products with the correct ingredients, however with fake packaging, with the wrong ingredients, without active ingredients or with insufficient active ingredients [1]. Their use represents a significant problem for public health because this can lead to therapeutic failure, drug resistance and could result in death. Consequently, anti-counterfeiting testing is intensifying and the problem is gaining relevance in literature [2–9].

The directive 2001/83/EC of The European Parliament and of The Council [10] (amended by Directive 2004/27/EC) not only defines medicinal products, but also homeopathic medicinal products which are consequently subjected together with others, to respect manufacturing and labelling regulations.

The Italian National Institute of Health, is involved in the research, quality, safety and efficacy of pharmaceuticals and has now initiated a study on the safety of homeopathic medicinal products. In fact, past evidence of cosmetics containing illegal substances on the market [11], has also prompted us to consider the presence of these substances in homeopathic preparations. Therefore, the aim of this study was to ascertain that synthetic active substances usually administered in traditional medicine were not added illegally in homeopathic products. In fact, homeopathic products should not contain these substances. For this purpose Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) were selected as the first pharmaceutical class to be investigated. Among the NSAIDs Naproxen, Ketoprofen, Ibuprofen, Piroxicam, Diclofenac, Nimesulide and Paracetamol (although the anti-inflammatory effect of Paracetamol is weak compared to its analgesic and antipyretic effects) were chosen as the most frequently utilised in allopathy. As far as we are aware, there is no literature on counterfeit homeopathic products. There is also no literature on the simultaneous separation and detection by UV and ESI-MS of the seven active substances that we have investigated. Numerous papers have

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been published about the analysis of NSAIDs in a variety of matrixes with different chromatographic techniques. Among them gas chromatography–mass spectrometry (GC–MS) [12–17] has been extensively utilised even though it often requires analyte derivatisation. HPLC is fast, sensitive and has also been utilised with UV [18–35] and with fluorescence [36,37] detection. Capillary electrophoresis (CE) [38,39] and CE–MS [40,41] have also been utilised. The HPLC–MS technique is increasingly being used in control laboratories because it is simple, fast and highly sensitive. Furthermore, it provides a high degree of specificity and additional information about the structure of the analytes. The analysis of NSAIDs with HPLC–MS has been reported [18,19,21,32,42–57] with both ESI or atmospheric pressure chemical ionisation (APCI) [21,53,55,56] sources. In this study a simple HPLC separation method with both UV and ESI-MS detection to investigate the illegal presence of NSAIDs in homeopathic preparations was developed. It was hypothesised that in order to produce a pharmaceutical effect an illegal product should contain an amount of these substances comparable to the minimum of these active substances usually administered in allopathy. If an illegal product contains NSAIDs at this level of concentration, UV detection is normally employed. However, to detect these substances in an illegal product containing one or more NSAIDs at lower concentrations and in order to have a mass confirmation of their presence, the use of ESI-MS is more suitable.

For this purpose diverse homeopathic mother tinctures, solutions, granules, tablets, creams and suppositories which are commonly prescribed in inflammation cases were chosen and utilised to test our method.

2. Experimental

2.1. Chemicals

The pharmaceutical standards of Diclofenac sodium salt (purity >99%), Ketoprofen (purity 99.7%), Nimesulide (purity >98%), Naproxen (purity >98%) and Piroxicam (purity >99%) were supplied by Sigma (Milan, Italy); Paracetamol (purity >98%) by Ciba (Torre Annunziata, Italy); Ibuprofen (purity 99%) by E. Pharm (Trento, Italy); benzoic acid (purity 99.5%) by Carlo Erba (Milan, Italy).

HPLC grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Acetic acid and HiPerSolv Water for HPLC were purchased from BDH (Poole, England).

Different brands of homeopathic preparations were used. Mother tinctures: 35% hydroalcoholic solution of *Echinacea angustifolia*; 65% hydroalcoholic solution of *Hieracium pilosella*; 65% hydroalcoholic solution of *Plantago major*. Solutions: Lachesis (030 LM); Sulphur (030 LM); *Atropa belladonna* (D2). Tablets: Bryonia. Creams: *Calendula officinalis*. Suppositories: *Calendula officinalis*. Granules: *Aconitum napellus*; Cantharis.

2.2. Instrumentation

HPLC–ESI-MS analyses were conducted using an Agilent Technologies (Palo Alto, CA, USA) HPLC–ESI-MS system. ESI-MS was utilised as it is commonly reported as being suitable for the detection of the chosen pharmaceuticals [18,19,32,42,43,46–51,54]. The 1100 series HPLC system consisted of a binary pump, a degasser, an autosampler with thermostat control, a column compartment with thermostat control and a diode-array UV detector. The ESI source was connected to a single quadruple mass spectrometer SL model. The Agilent ChemStation software Rev. A.08.04 (1008) was used for the data processing and control of the HPLC–ESI-MS system. The analytical column used for chromatographic separations was Agilent Zorbax Eclipse XDB C-18 (150 mm × 4.6 mm, 5 μm). HPLC vials and PTFE screw caps were purchased from Agilent Technologies. Samples were filtered by Acrodisc syringe filters, pore size 0.2 μm.

Table 1
Homeopathic preparations analysed, typical daily dosage and quantity analysed

Homeopathic preparation	Daily dosage	Quantity analysed
Cream	1 g twice a day	600 mg
Suppository	1 suppository twice a day	500 mg
Mother tincture	20 drops three times a day	0.5 mL
Solution	20 drops three times a day	0.5 mL
Tablet	5 tablets a day	500 mg
Granule	5 granules every 2 h	600 mg

2.3. Standard solutions and sample preparation

Benzoic acid was utilised as the internal standard (IS) and multicomponent standard stock solutions were prepared in methanol at 1 mg mL^{-1} mass concentration. For UV analysis, working standard solutions were prepared in the 50–400 μg mL^{-1} range (50, 100, 200, 250, 300 and 400 μg mL^{-1}) by appropriate dilution of stock solutions with methanol. For MS analysis they were prepared in the 0.1–50 μg mL^{-1} range (0.1, 0.2, 1, 2, 4, 10, 20, 25, 40 and 50 μg mL^{-1}).

For recovery measurements the amount shown in Table 1 for each homeopathic preparation was added with IS and spiked with standard stock solutions. Spiking was performed at two different levels for UV and ESI-MS and samples were brought to a final volume of 50 mL with methanol–water (80:20, v/v). For UV detection, solutions were spiked at 100 and 200 μg mL^{-1}. Creams and tablets were spiked at 10 and 20 μg mg^{-1}. Granules and suppositories were spiked at 8 and 17 μg mg^{-1}. For ESI-MS detection, solutions were spiked at 2 and 10 μg mL^{-1}. All the other homeopathic preparations were spiked at 0.2 and 1 μg mg^{-1}.

After spiking, mother tinctures and solutions were stirred in order to allow the standards to be homogeneously incorporated, sonicated for 10 min and then diluted to final volume. Granules and tablets were firstly powdered in a mortar, weighed and spiked; then they were dispersed in 10 mL of methanol–water (80:20, v/v), stirred, sonicated for 10 min and then brought to final volume. Creams were spiked, stirred in order to allow them to be homogeneously incorporated in the matrix and then solubilised with 10 mL of methanol; these were then stirred, sonicated for 10 min and diluted to final volume. Suppositories

Table 2
Name and chemical formula, relative molecular mass (M_r), retention time (t_r), base peak and fragmentor voltage for each active substance

Analyte	Formula	M_r	t_r (min)	Base peak	m/z	Fragmentor voltage (V)
Paracetamol	$C_8H_9NO_2$	151.2	2.5	$[M+H]^+$	152	120 (+)
Piroxicam	$C_{15}H_{13}N_3O_4$	331.4	16.6	$[M-H]^-$	330	110 (−)
Ketoprofen	$C_{16}H_{14}O_3$	254.3	19.9	$[M-H]^-$	253	80 (−)
Naproxen	$C_{14}H_{14}O_3$	230.3	20.1	$[M-H]^-$	229	70 (−)
Nimesulide	$C_{13}H_{12}N_2O_5S$	308.3	22.7	$[M-H]^-$	307	170 (−)
Diclofenac sodium salt	$C_{14}H_{10}Cl_2NNaO_2$	318.1	24.9	$[M-Na]^-$	294	80 (−)
Ibuprofen	$C_{13}H_{18}O_2$	206.3	25.6	$[M-H]^-$	205	80 (−)
Benzoic acid (IS)	$C_6H_6O_2$	122.1	10.4	$[M-H]^-$	121	100 (−)

were preliminarily left in a thermostatic water bath at 37 °C with 10 mL of methanol for 40 min, then spiked, vigorously stirred and brought to room temperature for 1 h; the supernatant was then quantitatively transferred and brought to final volume after rinsing the residue several times.

For the recovery, unspiked homeopathic preparations were used as blanks, prepared as reported for spiked samples without the addition of the standards. Blanks were preliminarily injected in order to ascertain the absence of any peak at the retention times of our analytes. A 10-fold dilution was performed for samples analysed by ESI-MS.

All homeopathic preparations analysed for inspection were prepared as reported for blanks, added with IS and injected. All samples were prepared in triplicate and filtered before injection.

2.4. HPLC conditions

The mobile phase used in chromatographic separations consisted of a binary mixture of solvents acetonitrile A, and water with acetic acid 0.1%, pH 3.16 B, at a flow rate of 1 mL min^{-1}. The elution was isocratic for 3 min 15:85 (A:B, v/v), then was brought to 30:70 (A:B, v/v) in 7 min and finally from 30:70 to 90:10 (A:B, v/v) in 20 min. After each run, the column was washed with A for 5 min and then conditioned for 10 min with the initial mobile phase. The column thermostat was kept at 30 °C and sample thermostat at 10 °C. For all samples, the injected volume was 5 μL. Retention time for each analyte is given in Table 2.

Preliminary UV analyses were performed at different wavelengths as specified in literature for each active substance [18–35]. This was done to select the appropriate wavelength for all pharmaceuticals in order to utilise a single wavelength detector.

2.5. ESI-MS conditions

The ions to be monitored in Selected Ion-Monitoring mode (SIM) and the mass operating parameters were selected in preliminary Flow Injection Analysis (FIA) experiments, without the chromatographic column, with a 1 mL min^{-1} flow rate, and solutions at 0.1 mg mL^{-1} mass concentration (5 μL injected). The A and B percentages used corresponded to those occurring at the gradient elution times for each standard (Table 2). In FIA experiments, full scan acquisitions were made over the (100–1000 *m/z*) range in both negative and positive ionisation. The best mass parameters resulted in the following: capillary voltage 4.0 kV for positive and negative ionisation mode; drying gas (nitrogen) flow 13.0 L min^{-1}; drying gas temperature 350 °C; 60 p.s.i. nebuliser pressure; fragmentor voltages used, as shown in Table 2.

Analyses were performed in SIM mode by selecting for each standard the ions shown in Table 2. Taking into account the elution times, the following starting points of acquisition were selected for SIM experiments: positive channel: t = 0–30 min, *m/z* 152; negative channel: t = 0–15 min, *m/z* 121; t = 15–19 min, *m/z* 330; t = 19–22 min, *m/z* 229, 253; t = 22–24 min, *m/z* 307, and t = 24–30 min, *m/z* 205, 294.

3. Results and discussion

3.1. HPLC

Among the samples we tested (solutions, mother tinctures, tablets, creams and suppositories) none contained any of the seven active substances under investigation.

The short isocratic elution and the linear gradient with a simple binary mixture permitted an excellent separation of the seven pharmaceuticals which differ in lipophilicity (Fig. 1). The use of acetic acid in the aqueous phase produced a well-shaped chromatographic peak and prevented the dissociation of the acidic pharmaceuticals during the chromatographic separation. The different signal responses shown by pharmaceuticals with UV and ESI-MS detection can also be observed in the same figure.

After preliminary analyses the 245 nm wavelength was selected because all the analytes showed a high value of molar absorptivity with the exception of Ibuprofen (λ_{max} = 227) [21]. Nevertheless, this was discarded because of a baseline drift and a lower molar absorptivity for Paracetamol and Ketoprofen.

3.2. ESI-MS

Preliminary FIA tests were performed in order to evaluate the influence of acetic acid on negative ionisation and the comparison of peak areas with and without acetic acid demonstrated that negative ionisation was not affected by it.

On the basis of FIA experiments (area of the most abundant extracted ion) negative ionisation was selected for all active

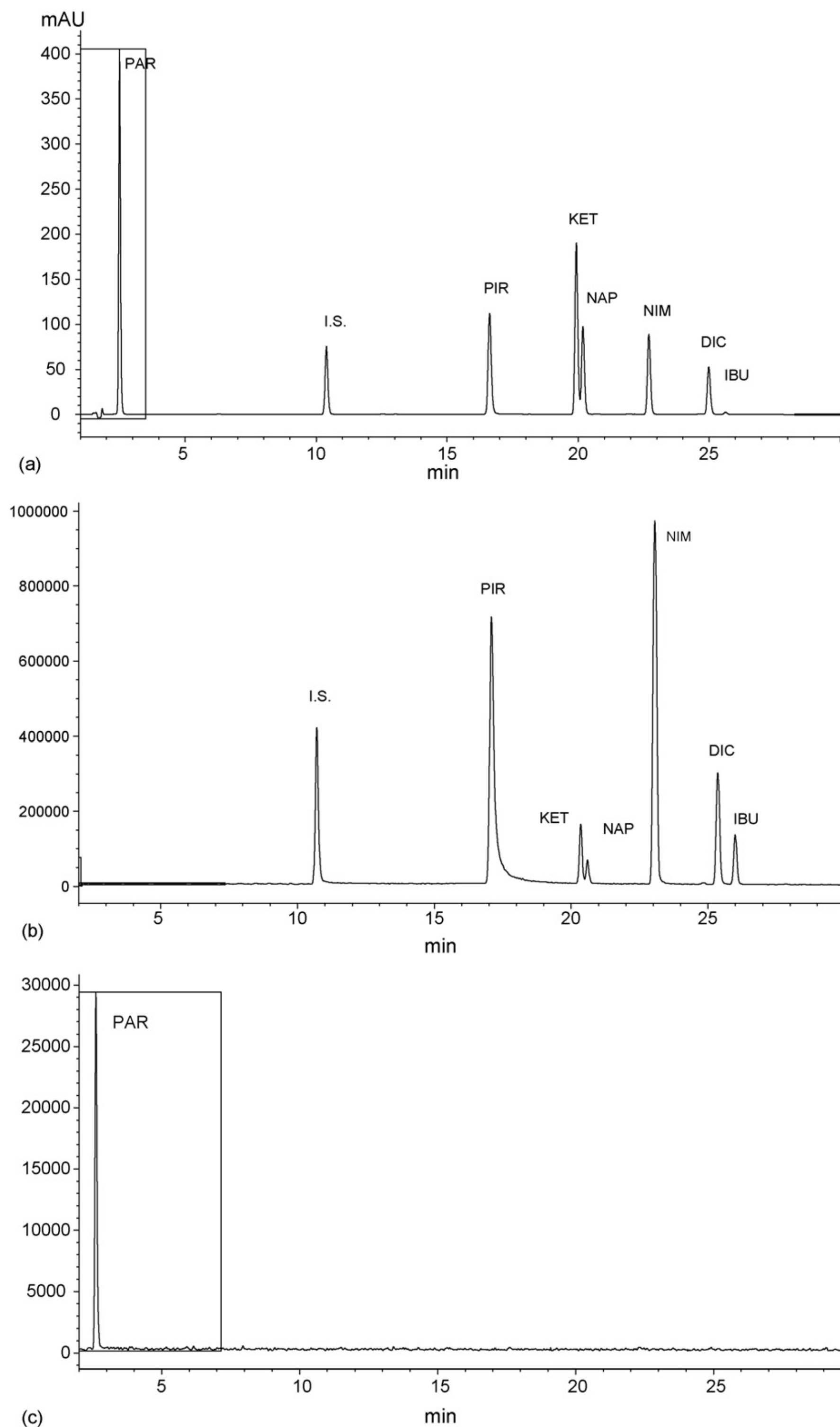

Fig. 1. Cream spiked by Paracetamol (PAR), Piroxicam (PIR), Ketoprofen (KET), Naproxen (NAP), Nimesulide (NIM), Diclofenac (DIC), Ibuprofen (IBU) and added by benzoic acid (IS); at 100 $\mu g\,mL^{-1}$ mass concentration, detected by UV at 245 nm (a); at 10 $\mu g\,mL^{-1}$ detected by ESI-MS in negative ionisation mode (b) and positive ionisation mode (c).

substances with the exception of Paracetamol which was ionised in positive mode. Even though Ketoprofen and Piroxicam were ionised in both positive and negative ionisation mode, a peak area of at least 10-fold or bigger, resulted in negative mode and also a lower fragmentation was evident. An intense deprotonated $[M-H]^-$ ion was observed for compounds in the negative mode, while a protonated $[M+H]^+$ ion was observed in positive mode. Consequently, the base peaks utilised for all standards in the quantitative analysis were $[M-H]^-$ in negative and $[M+H]^+$ in positive ionisation mode (Table 2).

Table 3
Linearity range; linear regression analysis ($y = ax + b$) of solution mass concentrations ($\mu g\, mL^{-1}$) (x) vs. ratios of peak to internal standard area (y); correlation coefficients; LOD and precision (R.S.D.%) for both UV and ESI-MS detection

Analyte	Range ($\mu g\, mL^{-1}$)		Linearity				Correlation coefficient	
	UV	MS	UV		MS		UV	MS
			a ($\times 10^{-3}$)	b	a ($\times 10^{-3}$)	b		
Paracetamol	50–400	10–50	33.4	0.0973	1.4	0.0147	0.999	0.956
Piroxicam	50–400	0.1–25	17.7	−0.1169	187.4	0.0881	0.999	0.995
Ketoprofen	50–400	1–25	24.6	0.0266	25.9	0.0006	0.999	0.999
Naproxen	50–400	2–50	12.1	0.0824	15.3	−0.0244	0.999	0.991
Nimesulide	50–400	0.1–25	12.7	0.0173	141.3	0.3291	0.999	0.993
Diclofenac	50–400	1–50	6.2	0.1373	57.1	0.095	0.979	0.993
Ibuprofen	50–400	1–50	0.4	−0.0045	22.6	6×10^{-6}	0.994	0.994

Analyte	LOD (ng)[a]		Precision (R.S.D.%)[b]				
	UV	MS	UV		MS		
			100[c]	400[c]	2[c]	20[c]	50[c]
Paracetamol	0.180	0.825	0.07	0.04		–	8
Piroxicam	1.150	0.160	1.48	1.35	7	1	–
Ketoprofen	0.600	0.750	1.43	1.33	9	4	–
Naproxen	0.625	1.000	0.09	0.13	3	–	5
Nimesulide	1.000	0.035	1.41	1.54	4	2	–
Diclofenac	1.500	0.345	0.15	0.20	5	–	4
Ibuprofen	41.50	0.380	0.22	0.09	9	4	–

[a] 5 μL injected.
[b] Percent relative standard deviation of six replicates.
[c] $\mu g\, mL^{-1}$.

3.3. Method validation

3.3.1. Linearity

Linearity of the method was studied in the 50–400 $\mu g\, mL^{-1}$ range by UV and in the 0.1–50 $\mu g\, mL^{-1}$ range by ESI-MS detection. Experimental data fitting was performed by linear regression analysis, plotting peak area ratios of each active substance to IS against the active substance mass concentration.

Good linearity was shown in all cases. Correlation coefficients were ($r^2 = 0.999$) for all analytes with the exception of Diclofenac ($r^2 = 0.979$) and Ibuprofen ($r^2 = 0.994$). With ESI-MS, $r^2 > 0.990$ was obtained for all analytes with the exception of Paracetamol ($r^2 = 0.956$). Equations and correlation coefficients are given in Table 3 for both detectors.

3.3.2. Precision

Precision was calculated on extracted samples and expressed as the relative standard deviation (R.S.D.%) of replicate measurements ($n = 6$) at two analyte mass concentrations for UV (100 and 400 $\mu g\, mL^{-1}$) and at three analyte mass concentrations for ESI-MS (2, 20 and 50 $\mu g\, mL^{-1}$). Good R.S.D.% values were obtained with both detectors. With UV lower R.S.D.% values were obtained for Paracetamol (00.7 and 0.04). With ESI-MS R.S.D.% ranged from 1 to 9. The higher values were observed at 2 $\mu g\, mL^{-1}$ mass concentration. Results are given in Table 3.

3.3.3. Recovery

The extraction recoveries of the analytes from matrixes were estimated using matrixes spiked with pharmaceutical standards at two levels for UV and ESI-MS (Table 4). The concentrations of the spiked samples were calculated from the calibration curves and compared to the theoretical values in order to calculate the extraction recoveries.

We hypothesised that in order to produce a pharmacological effect the amount of active substance added illegally should be comparable to the daily minimum amount administered in traditional medicine. The minimum daily dosages for Paracetamol, Piroxicam, Ketoprofen, Naproxen, Nimesulide, Diclofenac and Ibuprofen are 80, 20, 25, 250, 100, 50 and 200 mg, respectively. Consequently, an illegal homeopathic preparation should contain these amounts that can be detected with UV detector.

The amount of active substances spiked in samples analysed by ESI-MS detection was lower than the typical amount of active substances which were expected to give a significant pharmacological effect. Nevertheless, we investigated these concentrations to evaluate the hypothetical simultaneous presence of more than one NSAID, at a concentration lower than that normally used in allopathy.

Good recoveries were obtained for all analytes in different preparations. Slight differences were observed for the same analyte at the two mass concentrations with both detectors (Table 4).

3.3.4. LOD

The limit of detection (LOD) was beyond the scope of our study, as illegally added pharmaceuticals must be in a higher concentration range in order to produce a pharmacological

Table 4
Recoveries % ± standard deviation for each active substance in homeopathic preparations (%, mean ± S.D., $n = 3$)

Analyte	Solutions (μg mL^{-1})				Granules (μg mg^{-1})			
	UV		MS		UV		MS	
	100	200	2	10	8	17	0.2	1
Paracetamol	79 ± 3	80 ± 2	–	95 ± 5	78 ± 2	79 ± 2	–	109 ± 8
Piroxicam	90 ± 5	95 ± 4	98 ± 5	96 ± 3	92 ± 3	91 ± 3	93 ± 6	99 ± 4
Ketoprofen	94 ± 6	99 ± 4	100 ± 6	92 ± 6	100 ± 3	98 ± 3	77 ± 8	92 ± 8
Naproxen	89 ± 4	81 ± 3	121 ± 9	94 ± 7	81 ± 2	80 ± 2	100 ± 8	88 ± 5
Nimesulide	101 ± 5	99 ± 5	97 ± 7	93 ± 5	99 ± 2	85 ± 4	81 ± 3	84 ± 2
Diclofenac	79 ± 2	87 ± 4	65 ± 8	83 ± 5	79 ± 4	87 ± 4	52 ± 9	79 ± 7
Ibuprofen	74 ± 5	70 ± 3	96 ± 7	98 ± 6	67 ± 4	71 ± 3	79 ± 8	86 ± 6

Analyte	Tablets (μg mg^{-1})				Creams (μg mg^{-1})				Suppositories (μg mg^{-1})			
	UV		MS		UV		MS		UV		MS	
	10	20	0.2	1	10	20	0.2	1	8	17	0.2	1
Paracetamol	77 ± 4	79 ± 4	–	114 ± 8	77 ± 4	78 ± 3	–	119 ± 10	79 ± 6	80 ± 5	–	116 ± 9
Piroxicam	91 ± 7	94 ± 6	96 ± 6	102 ± 6	90 ± 4	94 ± 4	93 ± 6	108 ± 6	87 ± 8	92 ± 7	99 ± 9	106 ± 7
Ketoprofen	100 ± 6	98 ± 6	82 ± 7	94 ± 5	99 ± 5	98 ± 4	87 ± 5	102 ± 5	99 ± 8	99 ± 8	91 ± 8	103 ± 7
Naproxen	80 ± 4	81 ± 3	101 ± 6	93 ± 5	80 ± 6	81 ± 3	114 ± 7	104 ± 5	80 ± 6	81 ± 4	116 ± 9	103 ± 6
Nimesulide	99 ± 4	94 ± 3	77 ± 6	94 ± 4	98 ± 7	95 ± 7	97 ± 7	136 ± 7	95 ± 5	97 ± 3	104 ± 7	117 ± 5
Diclofenac	79 ± 2	87 ± 2	60 ± 9	77 ± 7	78 ± 5	87 ± 6	56 ± 8	95 ± 6	77 ± 6	86 ± 5	72 ± 8	82 ± 7
Ibuprofen	65 ± 3	69 ± 2	77 ± 5	95 ± 4	66 ± 6	70 ± 5	97 ± 9	115 ± 8	66 ± 7	85 ± 5	101 ± 11	113 ± 6

Quantity (μg) of standards spiked/mL (solutions) or mg (granules, tablets, creams and suppositories).

effect. Nevertheless, LODs defined as the minimum detectable amount of the analyte with a signal-to-noise ratio 3:1 were calculated.

Absolute detection limits (ng) are given for both detectors in Table 3. The LODs observed by UV detection were comparable to and sometimes better than those reported in literature. We obtained lower LODs for Paracetamol [20,30], Ketoprofen [23], Naproxen [25,33] and Nimesulide [34]. Furthermore, at 245 nm we obtained lower LODs than the ones obtained by ESI-MS for Paracetamol, Ketoprofen and Nimesulide.

In order to obtain lower LODs by ESI-MS it is possible to perform the acquisition with a single channel. In this case the positive channel can be switched off after the elution and detection of Paracetamol, and only then, to start the acquisition in negative ionisation mode. Nevertheless, with the exception of water analyses and analyses performed in tandem spectrometry [19,32,47,49,50,52–54] the LODs we obtained with ESI-MS are comparable to those reported in literature taking into account the low volume injected (5 μL). In particular, our LODs resulted lower than the ones reported in recent publications for Diclofenac [18,21,55], Ibuprofen [18,42], Piroxicam [21,56], Naprossene [21,42], Ketoprofen [55] and Paracetamol [57].

3.3.5. Specificity

The method showed a great specificity. With the exception of mother tinctures homeopathic preparations generally do not show problems of specificity due to their high dilution. The use of LC–ESI-MS is preferred in the analysis of mother tincture where a mass confirmation is necessary due to the presence of related substances of natural origin that can interfere.

4. Conclusions

The simple method developed in this study combining HPLC separation with UV and ESI-MS detection allowed a rapid screening of homeopathic formulations namely solutions, mother tinctures, tablets, granules, creams, and suppositories, in order to investigate the illegal presence of NSAIDs. A short isocratic elution and a simple linear gradient allowed the separation of the seven active substances. Good signal response was shown at 245 nm wavelength by UV and by ESI-MSI by all analytes, good linearity, and specificity was found in both detectors. The use of ESI-MS is preferred owing to its great specificity for the analysis of mother tinctures where the presence of related substances of natural origin could interfere in UV detection. LODs values obtained by UV and ESI-MS are comparable to those reported in literature. For Paracetamol, Ketoprofen and Naproxen the UV LOD values were lower than those performed by ESI-MS, even though LODs by ESI-MS can be improved as previously reported. However, none of the homeopathic preparations that we tested contained any of the seven active substances under investigation. Nevertheless, our goal is to investigate the presence of other classes of active substances.

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