Simultaneous Determination of Caffeine, Ergotamine, and Metamizol in Solid Pharmaceutical Formulation by HPTLC–UV-FLD with Mass Confirmation by Online HPTLC–ESI-MS

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Abstract

A new high-throughput method is developed to quantify caffeine, ergotamine, and metamizol in a solid pharmaceutical formulation. After dissolution, the compounds are separated on silica gel 60 F_{254} high-performance thin-layer chromatography (HPTLC) plates with ethyl acetate–methanol–ammonia 90:15:1 (v/v/v) as the mobile phase. Detection is performed by UV absorption at 274 nm for caffeine and metamizol, and by fluorescence at 313 /> 340 nm for ergotamine. Calibrations are linear or polynomial with determination coefficients (R^2) ≥ 0.9986. Recoveries of the three compounds are between 95% and 102% at three different concentration levels. Repeatability [relative standard deviation (RSD)] of all substances in the matrix is between ± 0.9% and ± 1.7%. Intermediate precision (RSD) of the three compounds range from ± 2.0% to ± 3.1%. Mass confirmation is performed by a single quadrupole mass spectrometry in positive electrospray ionization full scan mode for caffeine and ergotamine and in negative mode for metamizol. The results proved that this method is a simple and reliable alternative for routine analysis.

Introduction

Headache disorders are one of the most frequently reported symptoms and have been associated with impaired quality of life, increased incidence of depression, musculoskeletal pain, and disability (1). Epidemiologic studies have found that 57% of males and 76% of females had one or more headache attacks per month (2). Recent studies indicate that approximately 4 million men and 19 million women in the US population have migraine attacks (2). In the case of Europe on 1-year prevalence, 51% of adults indicated a headache attack (3), and among German adolescents, the 3-months prevalence was 69% (4). Pharmaceutical companies offer different kinds of analgesic and nonsteroidal anti-inflammatory drug mixtures, with or without ergot alkaloids and caffeine for acute headache therapy. Diverse combinations have been commercialized, mixing paracetamol (acetaminophen), aspirin (acetylsalicylic acid), or metamizol with caffeine and ergotamine. Several methodologies have been developed to determine these multi-component mixtures or to quantitate a single component. Among these, pharmaceuticals that contain metamizol, caffeine, or ergotamine, separately or in combination with other drugs, have been quantitated by spectrophotometry–UV (5–7) high-performance thin-layer chromatography (HPTLC)–UV (8–10), high-performance liquid chromatography (HPLC)–UV (11–15), capillary electrophoresis–UV (16,17), and flow injection analysis (18). However, no reference is available for the simultaneous determination of these three compounds by HPTLC or HPLC.

For hyphenation of HPTLC with mass spectrometry several techniques have been employed: direct analysis in real time (DART) (19), extraction via a special surface sampling probe followed by electrospray ionization (ESI) (20–22), desorption ESI (23), matrix-assisted laser desorption-ionization (MALDI) (24,25), fast atom bombardment (FAB) (26), continuous wave diode laser desorption (27), and IR laser desorption (28), as well as plunger-based extractors (29,30). Recently a modified plunger-based device enabling extraction from glass-backed plates (31) was well proven for confirmation of substances in the lower pg-range on silica gel phases (32,33). The latter approach was considered to be suitable for this study. The objective of this work was to develop a high-throughput analytical method to simultaneously detect caffeine, ergotamine tartrate, and metamizol in solid pharmaceutical formulations by HPTLC–UV–fluorescence detection (FLD) with mass confirmation via HPTLC–ESI-MS.

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Experimental

Reagents and samples

Caffeine (> 99%) was obtained from Fluka (Buchs, Switzerland), and metamizol-Na (> 99%) and ergotamine tartrate (> 97%) were from Sigma (St. Louis, MO). Ethyl acetate, methanol, and ammonia 25% were purchased from Merck (Darmstadt, Germany); all solvents were at least of analytical grade or distilled before use. Chromatography was performed on HPTLC glass-backed plates from Merck, coated with a 200-µm layer of silica gel 60 F_{254}. The pharmaceutical products of two different brand names were purchased in a Chilean pharmacy.

Standard solutions

For the ergotamine standard solution: 5 mg of ergotamine tartrate were accurately weighed into a 100-mL volumetric flask, dissolved, and diluted to volume with methanol–water 7:3 (v/v). This solution, when stored refrigerated and protected from light, was stable for 48 h.

For the caffeine-metamizol standard solution: 10 mg of caffeine and 20 mg of metamizol-Na were accurately weighed into a 100-mL volumetric flask, dissolved, and diluted to volume with methanol. This solution, under the same conditions as described previously, was stable for at least 5 days.

Sample preparation

Five tablets were weighed and grounded in a mortar. An accurately weighed fraction equivalent to one tablet (0.6 or 0.7 g depending on the brand name used, containing as active ingredients 1 mg ergotamine tartrate, 100 mg caffeine, and 300 mg metamizol-Na) was transferred to a 50-mL volumetric flask completely covered with aluminum foil and dissolved in 40 mL methanol–water 7:3 (v/v). The flask was shaken for 20 min in a KS 125 basic shaker (IKA, Staufen, Germany) operating at 500/min, sonicated for 10 min in a Sonorex Super RK106 ultrasonic bath (Bandelin, Berlin, Germany), and filled up with methanol–water 7:3 (v/v). An aliquot was filtered (0.45-µm pore size) and used for HPTLC analysis. For metamizol and caffeine analysis, the filtered solution was diluted 20 times with methanol. Both solutions, stored refrigerated and protected from light, were stable for at least 24 h.

Chromatography

Samples and standard solution were applied with an Automatic TLC Sampler IV (ATS IV) from CAMAG (Muttenz, Switzerland), with the following settings for 11 tracks per plate: band length, 6.0 mm; track distance, 8 mm; band velocity, 120 nL/s; and first application, x axis and y axis, 10.0 mm each. Sample application volumes of 8 µL for ergotamine analysis and 2 µL for caffeine and metamizol analysis were used. Standard application volumes ranged from 0.5 to 6 µL.

Chromatography was carried out in a 10 × 10 cm flat bottom chamber (CAMAG) up to a migration distance of 80 mm using ethyl acetate–methanol–ammonia 90:15:1 (v/v/v) as the mobile phase. The chamber was saturated with mobile phase (without filter paper) for 15 min. After development, the plate was dried in a stream of warm air for 2 min. Optionally, chromatography could be performed in the automated developing chamber (ADC2, CAMAG).

The plate was scanned with the TLC Scanner 3 (CAMAG) with a slit dimension of 4.0 mm × 0.1 mm and a scanning speed of 100 mm/s. In absorption mode, caffeine and metamizol were measured at UV 274 nm, and in fluorescence mode, ergotamine was scanned at 313 /> 340 nm. All instruments were controlled via the software platform winCats 1.4.1 Planar Chromatography Manager (CAMAG). Statistical analysis was carried out with GraphPad Prism 4.0 software.

HPTLC–MS

After scanning the plate, the position of each compound was marked. Using an HPLC pump (HP 1100, Agilent Technologies, Palo Alto, CA) and the interface ChromeXtrakt from ChromAn (Holzhausen, Germany), the HPTLC plate was connected to the VG platform II single-quadrupole MS from Micromass (Manchester, UK). The compounds were online eluted from the layer with a mixture of methanol and formate buffer (10 mmol/L, pH 4.0) 19:1 (v/v) at a flow rate of 0.1 mL/min. The MS system was operated in the full scan mode with the following parameters for ESI^+: source temperature, 120°C; capillary voltage, 3.5 kV; HV lens, 0.5 kV; cone voltage, 55 V; dwell time, 0.5 s; inter channel delay, 0.02; repeats, 1; and span, 0.5. For ESI^-: source temperature, 120°C; capillary voltage, –3.5 kV; HV lens, 0.5 kV; cone voltage, –55 V; dwell time, 0.5 s; inter channel delay, 0.02; repeats, 1; and span, 0.5. Data were processed with Mass Lynx 3.2 software.

Results and Discussion

The new developed high-throughput method was based on simple HPTLC equipment (isocratic development, common plate material, reagents, etc.), which could be applied at any quality control laboratory. Because of the versatility of HPTLC regarding multiple detection, already proven for energy drinks in food analysis (34), the three compounds were detected after one single chromatographic run. In a second step, the reliability of online HPTLC–ESI-MS for confirmation of results was first shown for these compounds.

Mobile phase optimization and wavelengths selection

Using the systematic mobile phase optimization (35), three mobile phases were selected [i.e., dichloromethane–methanol–acetic acid (180:20:1, v/v/v), acetonitrile–ammonia (190:1, v/v), and ethyl acetate–methanol–ammonia (90:15:1, v/v/v)], whereby the latter resulted in the best separation. The optimal wavelengths of 274 nm for caffeine and metamizol and 250 nm for ergotamine were obtained by spectra recording. However, ergotamine was finally detected with increased sensitivity (by a factor of 8) and selectivity by combination of the fluorescence excitation at λ_{ex} 313 nm with the fluorescence emission λ_{em} > 340 nm (cut filter). Alternatively, also a 400 nm cut filter (already incorporated in the TLC Scanner 3) can be used; however, sensitivity was reduced by a factor of 3 if compared with the 340 nm cut filter.

Validation

Analytical response

For each compound, as recommended by the International

Committee on Harmonization (ICH) (36), a calibration plot was established with six analyte levels in duplicate, applying different volumes of the standard solution. Calibration plots of caffeine and metamizol showed polynomial regressions from 50 to 500 ng and from 100 to 1000 ng, respectively, both with determination coefficients (R^2) of 0.9999. The calibration plot of ergotamine showed a linear regression in the range of 50 and 300 ng with R^2 of 0.9986. For routine analysis, a three-point calibration was used, applying in duplicate the lowest, middle, and highest point of each calibration plot on the HPTLC plate.

Table I. Results From Recovery Analysis

	Amount added (μg/mL)*	Recovery† (%) (mean ± SD‡)
Caffeine	15	102 ± 0.9
	30	101 ± 0.8
	45	100 ± 2.3
Metamizol	45	101 ± 0.0
	90	101 ± 0.9
	135	100 ± 0.8
Ergotamine	3	95 ± 1.2
	6	97 ± 0.7
	9	98 ± 1.1

* Correspond to 15%, 30%, and 45% of the compound amount in sample solution.
† Mean of three determinations.
‡ Standard deviation.

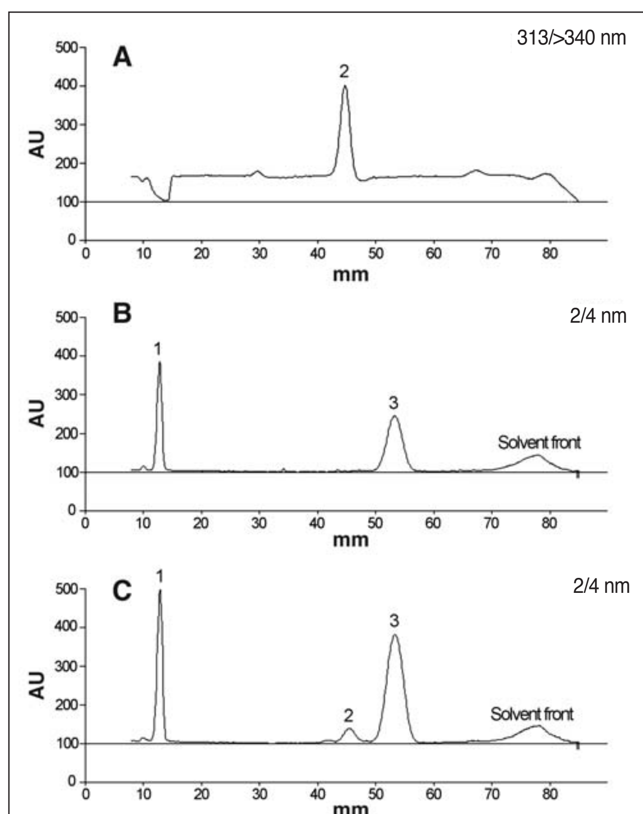

Figure 1. Chromatograms of the same sample track obtained by fluorescence (A) and absorbance (B) measurement showing: 600 ng/band metamizol, 1; 160 ng/band ergotamine, 2; and 200 ng/band caffeine, 3; Chromatogram of a standard track (1000 ng/band metamizol, 300 ng/band ergotamine and 500 ng/band caffeine) (C).

Accuracy and precision

Repeatability was determined analyzing the same sample (n = 6) on the same plate, showing a relative standard deviation (RSD) of ± 0.9% for metamizol, ± 1.7% for ergotamine, and ± 1.3% for caffeine. Intermediate precision of the three compounds was calculated measuring the same sample lot during three days in triplicate showing a RSD ± 2.0% for metamizol, ± 2.3% for caffeine, and ± 3.1% for ergotamine. Recovery was calculated spiking sample solutions with 15%, 30%, and 45% of the sample content, showing recoveries between 95% and 102% (Table I).

Limit of detection and quantitation

As recommended by the ICH (36), the detection and quantitation limits were calculated using a signal-to-noise ratio multiplied by 3 and 10, respectively. Considering an application volume of 10 μL, the detection and quantitation limits were 1.0 and 3.3 μg/mL (10 and 33 ng/band) for caffeine, 1.1 and 3.5 μg/mL (11 and 35 ng/band) for metamizol, and 0.6 and 2.0 μg/mL (6 and 20 ng/band) for ergotamine, respectively.

Robustness

During method development, several parameters were evaluated. The mobile phase composition should be freshly prepared, and the chamber should always be saturated for 15 min because the ammonia vapor proportion was highly relevant for a good resolution. The separation was successful if the aqueous ammonia ratio was within the RSD ± 20% [i.e., between 0.8 and 1.2 volume parts in the target mixture ethyl acetate–methanol–ammonia 90:15:1 (v/v/v)]. The migration distance had to be 80 mm to ensure a sufficient migration of metamizol out of the application zone. Even when the temperature and relative humidity mostly cannot be deliberately modified in planar chromatography, they are important aspects. The chromatographic selectivity was not influenced by either temperatures between 18°C and 27°C or relative humidities from 28% to 55%. Also, different plates lots from the same manufacturer did not alter the selectivity.

Samples analysis

Three different samples of two brand names were analyzed in duplicate. Figure 1 depicts typical sample chromatograms obtained by multiple wavelengths scanning. Table II shows that both brand names content fulfill the range between 90% and 110% stipulated by the United States Pharmacopeia (37) with regard to the ergotamine and caffeine tablets. An unknown impurity was found in all samples and separated from the active ingredients by the mobile phase. However, if the mobile phase polarity was increased (e.g., by adding more methanol) this impurity was coeluting with the caffeine band, increasing its value by 10%.

Compounds identification by HPTLC–MS

All sample compounds were online eluted from the HPTLC plate, ionized by ESI, and recorded in the positive and negative full scan ion mode. The mass spectra of the compounds was shown in the ESI^+ mode the $[M+H]^+$ ions for caffeine and ergo-

tamine at m/z 195 and 582 as well as the $[M+Na]^+$ ions at m/z 217 and 604, respectively. In the ESI^- mode, metamizol was detected at m/z 310 $[M–Na]^-$ (Figure 2). Small fragments in the spectra were caused by the layer material.

Table II. Results of Sample Analysis

	Amount labeled (mg)	Found* (%) (mean ± SD)
Brand I		
Caffeine	100	106 ± 2.9
Metamizol	300	103 ± 3.0
Ergotamine	1	105 ± 3.5
Brand II		
Caffeine	100	109 ± 3.4
Metamizol	300	98 ± 1.9
Ergotamine	1	97 ± 3.2

* Mean of three different samples.

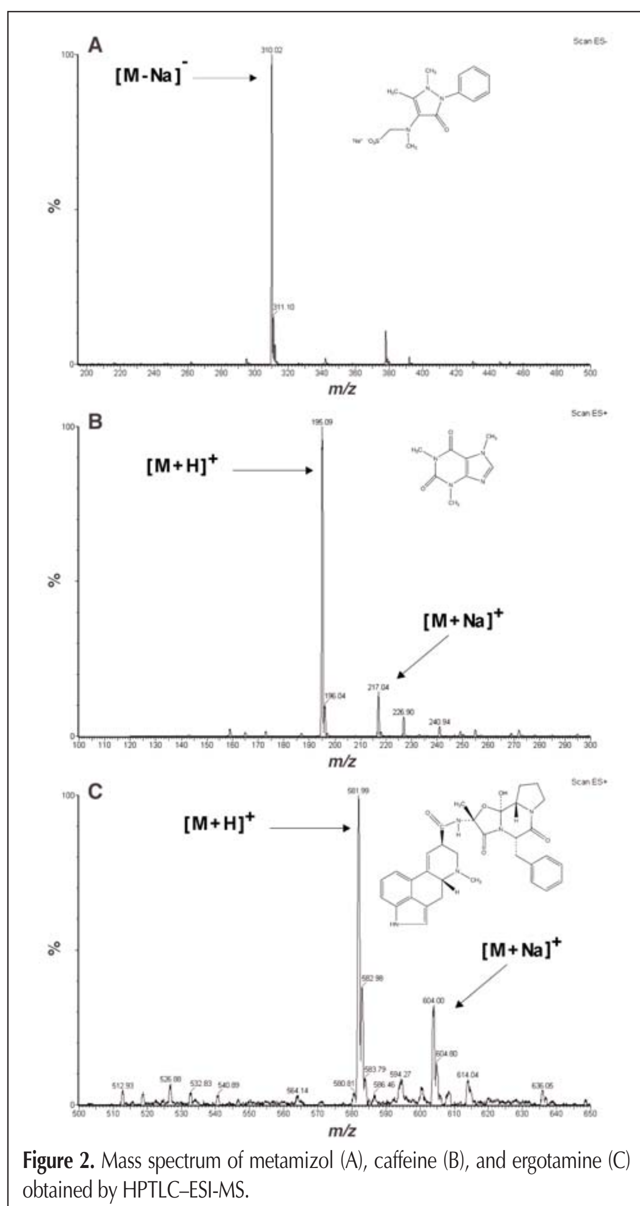

Figure 2. Mass spectrum of metamizol (A), caffeine (B), and ergotamine (C) obtained by HPTLC–ESI-MS.

Conclusion

In order to ensure the quality of the product, the pharmaceutical industry in particular needs fast, reliable, and low cost analytical methods. The validation data showed that this HPTLC method is a reliable high-throughput alternative for simultaneous measurement of caffeine, ergotamine, and metamizol. To the best of our knowledge, the online identification by HPTLC–ESI-MS of these three compounds was successfully achieved for the first time.

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