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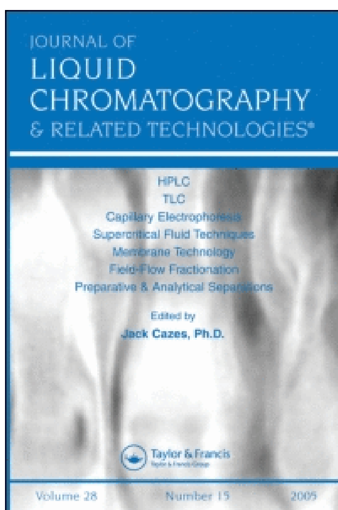

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QUANTIFICATION OF PYRITINOL IN SOLID PHARMACEUTICAL FORMULATION BY HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY-ULTRAVIOLET DETECTION AND SELECTIVITY EVALUATION BY MASS SPECTROMETRY

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QUANTIFICATION OF PYRITINOL IN SOLID PHARMACEUTICAL FORMULATION BY HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY-ULTRAVIOLET DETECTION AND SELECTIVITY EVALUATION BY MASS SPECTROMETRY

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□ *A high-throughput method for pyritinol quantification in solid pharmaceutical formulation was developed. Chromatography was performed on silica gel 60* F_{254} *HPTLC plates using the mixture dichloromethane – methanol – formic acid 9:1:1 (v/v/v) as mobile phase. Detection was carried out by UV absorbance at 300 nm. Calibration showed a polynomial regression with a determination coefficient (*R^2*) of 0.9992. For chromatography, repeatability (relative standard deviation, RSD) and intermediate precision (RSD) in matrix were 0.4% and 3.0%, respectively. Recoveries of spiked samples at three levels ranging from 98.5 to 101.9% with intermediate precisions of RSD 3.7 to 4.7%. Limits of detection and quantification were 0.6 and 2.0 µg mL*$^{-1}$ *(6 and 20 ng/band), respectively. The method capacity to detect degradation products and/or byproducts within routine conditions of analysis was evaluated through forced degradation processes. Selectivity was evaluated determining the peak purity by UV-spectrophotometry, which showed correlation coefficients (r) > 0.9997. Additionally, peak identity and purity was confirmed by mass spectrometry. The mass spectra showed just pyritinol ions at m/z 369* $[M+H]^+$ *and 391* $[M+Na]^+$ *being acquired directly from the sample bands by an elution-based interface. Considering the validation results, reduced analysis cost, accelerated analysis time, and high throughput capacity, this simple, yet reliable planar chromatographic method is a good alternative for pyritinol analysis in pharmaceutical formulations.*

Keywords high-performance thin-layer chromatography, mass spectrometry, planar chromatography, pyrithioxin, pyritinol

INTRODUCTION

Pyritinol or pyrithioxin (Fig. 1) structurally related to vitamin B_6 but without vitamin activity, is one of the oldest nootropic drugs used in more than 50 countries. According to the latest studies, between 2000 and 2004,

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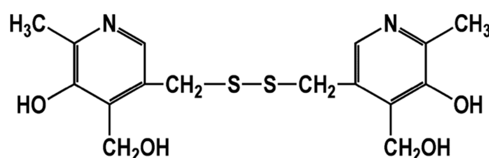

FIGURE 1 Pyritinol chemical structure (3,3′-[dithiobis(methylene)] bis [5-hydroxy-6-methyl-4-pyridinemethanol]).

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more than 100,000 individuals in the European Union consumed pyritinol[1] and an increase in the consumption is expected based on the demographic data of several countries. Since its patent by Merck, different pharmacological activities have been ascribed to pyritinol; some of these have been proven through successful clinical trials, e.g., in normal volunteers was observed an enhancement on their psychopharmacological performance,[2] in patients with probable Alzheimer's disease, pyritinol produced a higher response to stimulation tasks[3] and it was therapeutically effective in patients with mild to moderate senile dementia of the Alzheimer type.[4] Besides its intrinsic nootropic activity, other unusual uses have been reported for pyritinol, viz., it was highly effective in the treatment of patients with rheumatoid arthritis[5] and it showed scavenger activity over free radicals.[6] However, together with its beneficial properties, some serious adverse effects were attributed lately, i.e., cholestatic hepatitis[1] and acute pancreatitis.[7]

Through all these years, several analytical methods have been used to determine pyritinol in pharmaceutical formulations and other matrices, e.g., UV[8] and Vis spectrophotometry,[9] derivative spectrophotometry,[10] polarography,[11] ion-pair high-performance liquid chromatography (HPLC),[12] and conductimetry.[13] Thin-layer chromatography (TLC) has been used in combination with principal component analysis and other chromatographic techniques for simultaneous qualitative analysis of several drugs, including pyritinol.[14–16] Nevertheless, there was no reference available for quantification of pyritinol by planar chromatography.

In pharmaceutical analysis, TLC methods were, in general, substituted by HPLC methods without giving high-performance thin-layer chromatography (HPTLC) a real chance. This was criticized by experienced HPTLC experts.[17,18] Thus, the intention was to show that HPTLC, which stands for sophisticated instrumentation and enhanced separation power based on the reduced particle size of the adsorbent used, could be an alternative, or at least a complementary method, to HPLC. It is worth to follow such an intention because in the last decade the planar chromatographic system has continually been improved through full automatization of its single steps, including automated control of the plate activity and chamber

climate for highly reproducible developments. Consequently the reproducibility was severely increased accomplishing highly reliable quantitative analysis. Together with other well-known features, viz., multiple detection by UV/Vis and fluorescence,[19] *in situ* post-chromatographic derivatization,[20] and less extensive sample preparation than other chromatographic techniques,[21–24] planar chromatography seems to be an effective tool for high-throughput analysis. In addition, coupling of HPTLC with mass spectrometry (MS) has gained growing attention in the last decade and an overview is given in Morlock and Schwack.[25] Various desorption-based techniques have been reported for coupling, e.g., fast atom bombardment (FAB),[26] secondary ion mass spectrometry (SIMS),[27] matrix assisted laser desorption/ionization (MALDI),[28–30] desorption electrospray ionization (DESI),[31] electrospray-assisted laser desorption/ionization mass spectrometry (ELDI-MS),[32] continuous wave diode laser desorption/atmospheric pressure chemical ionization (LD-APCI),[33] direct analysis in real time (DART),[34] easy ambient sonic-spray ionization mass spectrometry (EASI-MS),[35,36] and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS).[37,38] Elution-based approaches include an automatic sampling surface probe coupled with electrospray ionization (ESI) by a micro-junction,[39] or elution-based interfaces coupled to APCI[40] or ESI.[41,42] The latter approach was successfully proven for qualitative[19,43,44] and quantitative analysis,[34,42] and, hence, it was used in this work. The objective of this work was to develop a quantitative high-throughput method for pyritinol determination in solid pharmaceutical formulation by HPTLC-UV-MS. In doing so, the identity of the separated zone and the satisfying separation power of HPTLC should be proven by purity calculation of UV-spectra and by HPTLC/ESI-MS spectra.

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EXPERIMENTAL

Reagents and Samples

Pyritinol dihydrochloride (>99%) was purchased from Sigma (Steinheim, Germany), methanol (HPLC grade), ammonium formate (>99%) and formic acid, were obtained from Fisher Scientific (Loughborough, UK). Dichloromethane was purchased from Acros Organics (Geel, Belgium). Ultra-pure water (18 MΩ * cm) was produced by Synergy System from Millipore (Schwalbach, Germany). Sodium hydroxide (≥99%) and hydrochloric acid (37–38%) were obtained from Merck (Darmstadt, Germany). Chromatography was performed on 10 × 10 cm HPTLC plates from Merck, coated with a 0.2 mm of silica gel 60 F_{254}. The pharmaceutical samples (different lots) were purchased in Chilean pharmacies.

Standard Solution

Pyritinol dihydrochloride was prepared in methanol for a given concentration of $0.1\,mg\,mL^{-1}$. This solution, stored refrigerated and protected from light, was stable for at least 5 days.

Sample Preparation

Five tablets were weighed and ground in a mortar. A fraction equivalent to one tablet (~0.5 g, containing 200 mg of pyritinol) was accurately weighed and transferred to a 250 mL Erlenmeyer flask, completely covered with aluminum foil. Pyritinol extraction was performed with 80 mL of methanol – water (7:3, v/v) in a KS 125 basic shaker from IKA (Staufen, Germany) at 500 (min^{-1}) for 20 min. The suspension was transferred to a 100 mL volumetric flask and filled up with methanol – water (7:3, v/v). An aliquot was filtered (0.45 µm pore size) and diluted 1:20 times with methanol prior to HPTLC analysis. This diluted solution, stored refrigerated and protected from light, was stable for at least 3 days.

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Chromatography

Samples and standard solution were applied by means of Automatic TLC Sampler 4 (ATS 4) from CAMAG (Muttenz, Switzerland), with the following settings for 12 tracks per plate: band length 4.0 mm, track distance 7.2 mm, dosage speed $150\,nL\,s^{-1}$, application position x-axis 8.0 mm and y-axis 10.0 mm each. Sample and standard application volumes were 3 µL and from 0.5 to 5 µL, respectively. Chromatography was carried out in a 10×10 cm twin trough chamber (CAMAG) using 5.5 mL dichloromethane – methanol – formic acid 9:1:1 (v/v/v) as mobile phase, up to a migration distance of 50 mm. After development, the plate was dried in a stream of warm air for 1 min. Detection was performed with TLC Scanner 3 (CAMAG) in UV-absorption mode at $\lambda = 300$ nm with a slit dimension of $3.0\,mm \times 0.1\,mm$ and a scanning speed of $20\,mm\,s^{-1}$. All instruments were controlled via software platform winCats 1.4.2 Planar Chromatography Manager (CAMAG). Statistical analysis was carried out with GraphPad Prism 4.0 software (San Diego, CA, USA).

Mass Spectrometry

The complete system setup has been described in details elsewhere.[42] Briefly, after scanning the plate, the pyritinol bands position was marked. Using an HPLC pump HP 1100 from Agilent (Palo Alto, CA, USA) and

the modified[45] plunger-based extractor ChromeXtrakt from ChromAn (Holzhausen, Germany), the HPTLC plate was online coupled to the electrospray ionization interface of the VG platform II single-quadrupole mass spectrometer from Micromass (Manchester, UK). The compound was eluted from the sorbent with a mixture of methanol and ammonium formate (10 mmol L^{-1}, pH 4.0) 19:1 (v/v) at a flow rate of 0.1 mL min^{-1}. 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 voltage 0.5 kV and cone voltage 55 V. Data were processed with Mass Lynx 3.2 software from Micromass.

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RESULTS AND DISCUSSION

Mobile Phase Optimization and Wavelength Selection

Following a systematic approach for mobile phase optimization,[46] three mobile phases were selected, i.e., acetonitrile – water (8:2, v/v), ethyl acetate – methanol (9:1, v/v) and dichloromethane – methanol (9:1, v/v). Due to peak tailing, each mobile phase was assayed with one part (about 10%) of acetic acid, formic acid or ammonia (25%). The best separation and chromatographic behavior was obtained with dichloromethane – methanol – formic acid (9:1:1, v/v/v). The intrinsic blue fluorescence of pyritinol, when irradiated at UV 366 nm, was lost in the acid medium, whereas it was conserved in alkaline ambience. Nevertheless, the acid medium was preferred because detection by UV-absorbance was still adequate. The optimal wavelength at 300 nm was ascertained by recording the pyritinol spectra (Fig. 2).

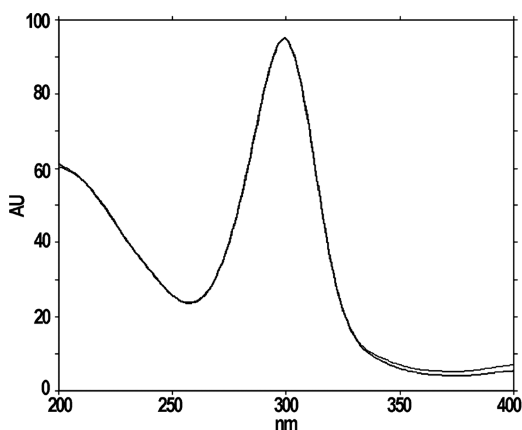

FIGURE 2 UV-spectra comparison of pyritinol standard (300 ng) and sample (300 ng), showing a correlation coefficient (r) > 0.9998.

Validation

Following the International Committee on Harmonization (ICH) guidelines,[47] a calibration plot was established with six analyte levels in duplicate (according to the data pair method), applying different volumes of the standard solution (Fig. 3). Pyritinol calibration data fit a polynomial regression model with a determination coefficient (R^2) of 0.9992 (Table 1). For routine analysis, a three-point calibration was used, applying in duplicate the lowest, middle and highest point of the calibration plot on both sides of the HPTLC plate (Fig. 4). Precision was evaluated through repeatability and intermediate precision. Repeatability of chromatography was calculated measuring the same pharmaceutical sample ($n = 6$) on the same plate, showing a relative standard deviation (RSD) of 0.4%. Intermediate precision of chromatography was determined analyzing the same sample during three days in sextuplicate on different plates, showing a RSD of 3.0%.

In order to evaluate the entire procedure, the recovery and the intermediate precision over the whole analysis system were calculated spiking ground samples, equivalent to one tablet (200 mg of pyritinol), with

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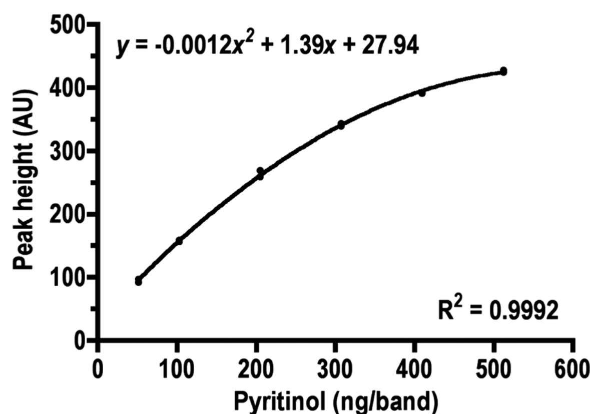

FIGURE 3 Pyritinol calibration plot. Each calibration level was determined in duplicate according to the data pair method (12 data points). Polynomial regression equation is described as $y = (ax^2 + bx + c)$.

TABLE 1 Pyritinol Calibration Established by Peak Height

Compound	Range (ng/band)	Equation $y = ax^2 + bx + c$	95% Confidence interval a	95% Confidence interval b	95% Confidence interval c
Pyritinol	50–500	$y = -0.0012x^2 + 1.39x + 27.94$	−0.0013 to −0.0011	1.32 to 1.46	20.05 to 35.83

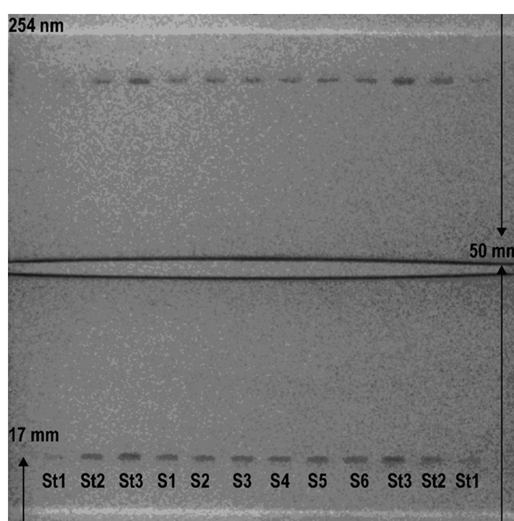

FIGURE 4 Pyritinol routine analysis on both sides of the HPTLC plate silica gel 60 F_{254}. S1 to S6 are pharmaceutical samples (3 μL, ~300 ng/band pyritinol), St1 to St3 are pyritinol standard levels of 50, 300 and 500 ng/band, respectively.

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pyritinol standard powder (>99%) at three different concentration levels. Each level, corresponding to 20, 40 and 60% of the labeled content, was prepared daily during 3 days and measured in duplicate. Recovery at three levels ranged between 98.5–101.9% (Table 2). Combined recovery ($n = 9$) showed a mean value of 100.3% with a standard deviation (SD) of 4.0%. Mean recovery evaluated with Student's t-test (two tails at 95% of confidence) did not show a significant difference with the true value ($t = 0.2323$, $df = 8$, $P = 0.8221$), therefore no correction factor for the recovery was applied. From these experiments, the intermediate precision for the entire procedure and for different concentration levels can be deduced to be within 4.7% (Table 2).

TABLE 2 Recovery Analysis Using Pyritinol Standard Powder

Amount Added (mg)	Amount Added (%[a])	Recovery Mean[b] ± SD[c](RSD) (%)
40	20	98.5 ± 3.6 (3.7)
80	40	101.9 ± 4.8 (4.7)
120	60	100.6 ± 4.5 (4.5)

[a]Percentage of the content labeled.
[b]Mean of three different determinations on different plates and days.
[c]Standard deviation and relative standard deviation (RSD).

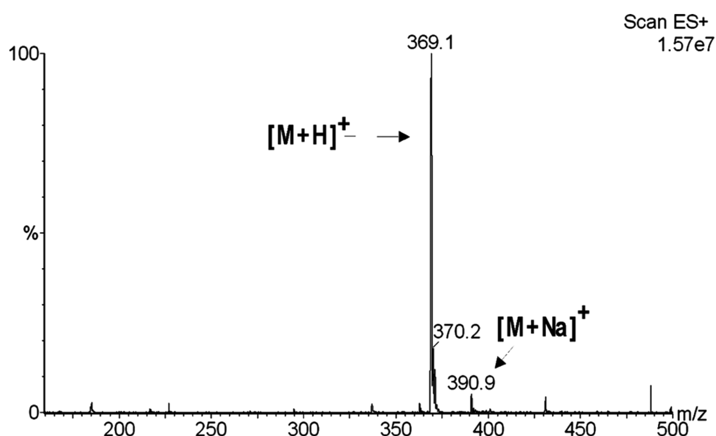

FIGURE 5 Mass spectrum of a pyritinol sample zone (300 ng/band) obtained by HPTLC/ESI-MS.

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Detection and quantification limits were calculated using signal to noise ratios (S/N 3 and 10, respectively). Considering an application volume of 10 µL, the detection and quantification limits were 0.6 and 2.0 µg mL^{-1} (6 and 20 ng/band), respectively. For lowering the detection limit, higher application volumes can be chosen, however 10 µL were highly adequate in this case.

Regarding robustness, the mobile phase should be freshly prepared, which is obvious due to its composition. Formic acid was crucial for a good chromatographic performance, and volume parts from 0.8 to 1.2 in the mobile phase led to successful separations. Lower acid volumes reduced the pyritinol retardation factor (R_F) and higher ones produced only a marginal R_F increase. In order to prevent peak tailing other acids, e.g., acetic acid, should not be used instead of formic acid. The increase of the methanol volume was directly related with the pyritinol R_F values, but also with an increase of peak tailing. Varied migration distances as well as different plate lots from the same manufacturer did not influence pyritinol quantification. Temperature and relative humidity are potential factors of influence in planar chromatography, temperatures between 18°C and 24°C, and relative humidities from 28% to 60% had not influence on the chromatographic performance.

Even though ICH describes the term specificity, which can be considered as special case of 100% gain in selectivity, due to general agreement and IUPAC recommendation, the preferred and promoted term is selectivity. Compound identification was established by R_F and UV-spectra comparison with the standard (Fig. 2). Selectivity was evaluated determining the peak purity. The correlation (r) of the UV-spectra within a peak between peak start and peak maximum was >0.9999 and between peak maximum

and peak end was >0.9997. Thus peak purity was assured in the UV-range. A further proof of adequate selectivity and separation power was given by MS. Through the elution-based interface sample bands were directly eluted from the plate into the mass spectrometer without any post-chromatographic protocol. The mass spectrum acquired in the m/z range from 160 to 500, showed only the protonated molecule of pyritinol at m/z 369 $[M+H]^+$ and its sodium adduct at m/z 391 $[M+Na]^+$ without any other relevant fragments (Fig. 5). Thus, the bands identity and an adequate chromatographic selectivity were confirmed. This was achieved even when using a circular elution-head, which was demonstrated to capture also adjacent zones, if any present. Whereas a band-shaped elution-head was demonstrated to be better sized and more selectively eluting band-shaped zones of interest[48] as given for this study.

Sample Analysis

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Two lots of pyritinol samples were analyzed in sextuplicate on different plates. Figure 6(A) depicts a typical chromatogram obtained by routine analysis at $\lambda = 300\,nm$. Table 3 shows that the content of both lots fulfill the typical range between 90% and 110% stipulated for several pharmaceutical tablets by the United States Pharmacopeia.[49] The presence of unknown impurities was evaluated applying the sample extraction solution without dilution (~2 µg of pyritinol). No impurities were observed in the chromatogram of those samples compared with the standard chromatogram at the same concentration. The excipients used in this pharmaceutical formulation are molecules like lactose, cellulose, etc., which lack of relevant chromophore groups and do not interfere with the evaluation in the UV-range.

Stability-Indicating Capability

The method capacity to detect degradation products and/or byproducts within routine conditions of analysis was evaluated through forced degradation processes. 10 mL aliquots of $1\,mg\,mL^{-1}$ pyritinol solution were mixed with 10 mL of $0.5\,mol\,L^{-1}$ HCl, 10 mL of $0.1\,mol\,L^{-1}$ NaOH or 10 mL of aqueous hydrogen peroxide solution (30%, v/v). The mixtures were heated at 60°C for 60 minutes. The pyritinol molecule (peak 1) suffered a major degradation when subjected to the three stressing conditions (Fig. 6 b–d). One degradation product (peak 2) was observed under acidic and another (peak 3) under alkaline conditions. Two degradation products were generated when hydrogen peroxide was present (peaks 4 and 5).

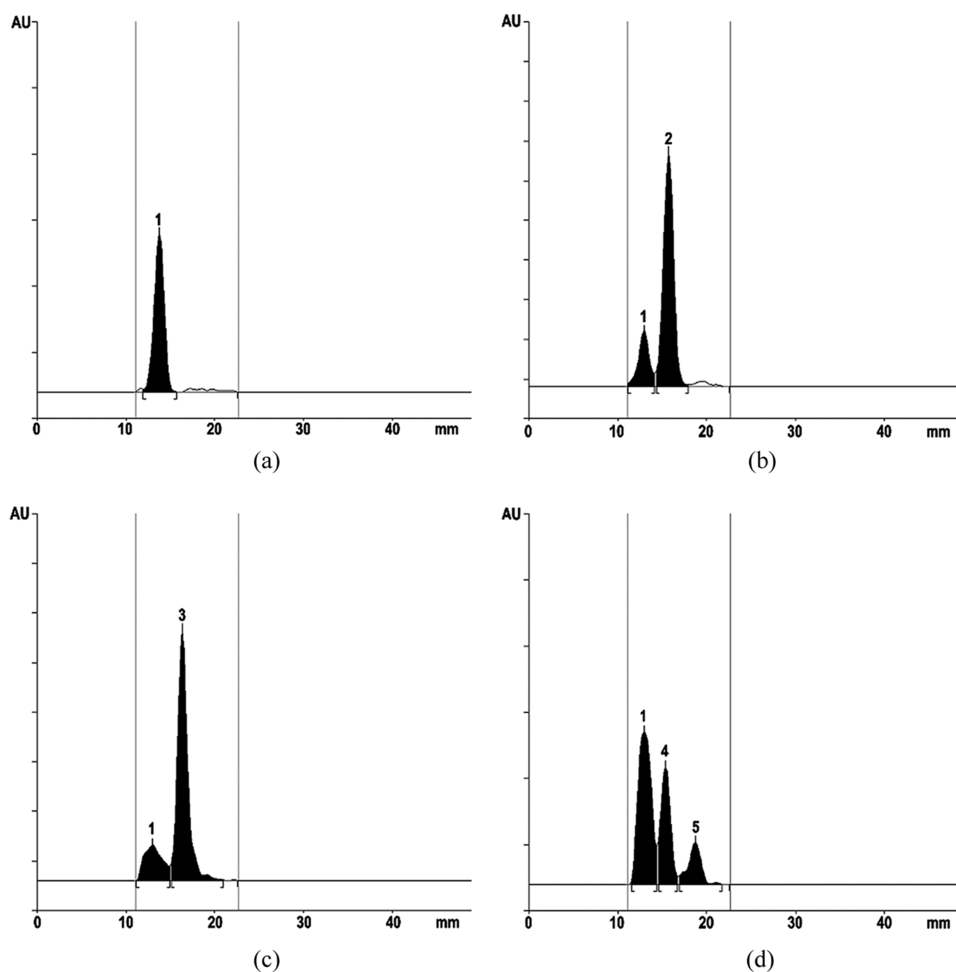

FIGURE 6 Chromatograms of pyritinol and its degradation products. (a) Pyritinol chromatogram of routine analysis. (b), (c) and (d) are pyritinol chromatograms obtained after forced degradations under acid, alkali and oxidative conditions, respectively. Peak 1: pyritinol. Peak 2: degradation product under acidic conditions. Peak 3: degradation product under alkaline conditions. Peaks 4 and 5: degradation products under oxidative conditions.

TABLE 3 Results of Sample Analysis

Sample Lot	Amount Labeled (mg)	Amount Found Mean[a] ± SD[b] (mg)
04092883	200	203.5 ± 2.4
05047635	200	197.8 ± 2.7

[a]Mean of the same sample measured in sextuplicate.
[b]Standard deviation.

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Figure 6 showed that the newly developed method allowed detecting any degradation product, if present, during routine analysis.

High-Throughput Capacity

Using this validated method, 6 samples (12 tracks, including 3 standards in duplicate) were simultaneously analyzed in less than 25 min on a 10×10 cm plate from one side. In other words, this meant a 'sample run time' of 2.1 min at a solvent consumption of 0.4 mL/sample. This good efficiency data were still improved when doubling the sample throughput by development from both plate sides (Fig. 4).

An even higher sample throughput and cost-reduction can be obtained by using a plate size of 20 cm $\times$ 10 cm. Compared to the 10×10 cm twin trough chamber, the 20×10-cm twin trough chamber (CAMAG) offered a 1-cm wider vapor space width, and chamber saturation was now required for separation. The twin trough chamber was saturated with 20 mL of mobile phase, using wetted filter paper on both sides, and the Horizontal Development Chamber (CAMAG) with 17 mL of mobile phase placed in the chamber bottom, apart from 4 mL on each side for chromatography. Both chamber saturations were done for 20 min, being essential to conserve the chromatographic behavior accomplished in the 10×10 cm twin trough chamber without any saturation. On the 20×10 cm plate, 18 samples (24 tracks, including 3 standard levels in duplicate) were analyzed in 31 min. In other words, this meant a 'sample run time' of 1.3 min at a solvent consumption of 0.8 mL/sample. Using development from both sides in the Horizontal Development Chamber, 36 samples (48 tracks, including 3 standards in duplicate on each side) were determined in 53 min. Again, in other words, this meant a 'sample run time' of 1.1 min at a solvent consumption of 0.5 mL/sample.

Regarding HPLC methods, pyritinol retention times were 2.9 min[12] at flow rates of 1.8 mL min^{-1} and 5.0 min[50] at flow rates of 1 mL min^{-1} using 4-min and 8-min runs, respectively. Therefore, 7.2 mL and 8 mL of mobile phase were required for the respective runs. Comparing the mobile phase volumes per sample run, the required HPTLC volumes were in the worst case by a factor of 9 (0.8 vs. 7.2 mL/sample) and in the best case by a factor of 20 (0.4 vs. 8.0 mL/sample) lower than the required HPLC volumes. Considering that both methods have approximately similar asset costs, the running costs of the newly developed HPTLC method were clearly reduced demonstrated simply on the mobile phase costs, and the gain is even better having in mind disposal costs of <0.01 Cent per sample and by up to another factor of 5 reduced stationary phase costs. Additionally, the HPTLC analysis times per 'sample run' were in the worst case by a

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factor of 2 (2.1 vs. 4 min) and in the best case by a factor of 7 (1.1 vs. 8 min) reduced compared to HPLC.

CONCLUSION

Quality control laboratories appreciate fast, reliable and low cost analytical methods. Considering the validation results, throughput capacity and analysis costs, this HPTLC method could be considered as a good alternative for pyritinol analysis in solid pharmaceutical formulations. Pyritinol identification by HPTLC/ESI-MS was successfully achieved for the first time using an universal (integration in all common HPLC-MS systems), versatile (options for further couplings, e.g., with NMR or ATR-FTIR[51]), plug & play (integration via two fittings) HPTLC-MS interface. The separation power of HPTLC can be proven to be adequate by the purity of mass spectra or by the purity correlation of UV-spectra. This new high-throughput method for pyritinol quantification was developed with common planar chromatographic equipment using regular reagents and could be employed at any quality control laboratory.

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REFERENCES

1. Maria, V.; Albuquerque, A.; Loureiro, A.; Sousa, A.; Victorino, R. Lesson of the week – Severe cholestatic hepatitis induced by pyritinol. Brit. Med. J. **2004**, *328* (7439), 572–574.
2. Hindmarch, I.; Coleston, D.M.; Kerr, J.S. Psychopharmacological effects of pyritinol in normal volunteers. Neuropsychobiology **1990**, *24* (3), 159–64.
3. Heiss, W.D.; Kessler, J.; Slansky, I.; Mielke, R.; Szelies, B.; Herholz, K. Activation PET as an instrument to determine therapeutic efficacy in Alzheimer's disease. Ann. N.Y. Acad. Sci. **1993**, *695*, 327–331.
4. Fischhof, P.K.; Saletu, B.; Ruther, E.; Litschauer, G.; Moslinger-Gehmayr, R.; Herrmann, W.M. Therapeutic efficacy of pyritinol in patients with senile dementia of the Alzheimer type (SDAT) and multi-infarct dementia (MID). Neuropsychobiology **1992**, *26* (1–2), 65–70.

5. Lemmel, E.M. Comparison of pyritinol and auranofin in the treatment of rheumatoid arthritis. The European Multicentre Study Group. Brit. J. Rheumatol. **1993**, *32* (5), 375–382.
6. Pavlik, A.; Pilar, J. Protection of cell proteins against free-radical attack by nootropic drugs: Scavenger effect of pyritinol confirmed by electron spin resonance spectroscopy. Neuropharmacology **1989**, *28* (6), 557–561.
7. Straumann, A.; Bauer, M.; Pichler, W.J.; Pirovino, M. Acute pancreatitis due to pyritinol: An immune-mediated phenomenon. Gastroenterology **1998**, *115* (2), 452–454.
8. Hassan, S.M.; El-Ashry, S.M.; El-Kerdawy, M.M. Spectrophotometric determination of pyrithioxin in pharmaceutical preparations. Spectrosc. Lett. **1990**, *23* (10), 1273–1284.
9. Sastry, C.S.P.; Krishna, D.M. Spectrophotometric determination of pyrithioxine in pharmaceutical formulations. Anal. Lett. **1995**, *28* (7), 1197–1207.
10. Shehata, M.A.; El-Sayed, M.A.; El-Bardicy, M.G.; El-Tarras, M.F. Stability-indicating methods for determination of pyritinol dihydrochloride in the presence of its precursor and degradation product by derivative spectrophotometry. J. AOAC Intl. **2005**, *88* (1), 80–86.
11. Belal, F. Anodic polarographic behavior and determination of pyrithioxin. Acta Pharm. Jugosl. **1991**, *41*, (2), 129–135.
12. Belal, F. Ion-pairing high-performance liquid chromatographic determination of pyridoxine and pyrithioxin in dosage forms. Anal. Lett. **1989**, *22* (8), 1897–1907.
13. El-Ashry, S.M.; Shehata, I.A.; El-Sherbeny, M.A.; El-Sherbeny, D.T.; Belal, F. Conductimetric determination of some drugs acting on the central nervous system. Chem. Anal. (Warsaw) **2000**, *45* (6), 859–866.
14. Daldrup, T.; Susanto, F.; Michalke, P. Combination of TLC, GLC (OV 1 and OV 17) and HPLC (RP 18) for a rapid detection of drugs, intoxicants and related compound. Fresenius J. Anal. Chem. **1981**, *308* (5), 413–427.
15. Musumarra, G.; Scarlata, G.; Cirma, G.; Romano, G.; Palazzo, S.; Clementi, S.; Giulietti, G. Qualitative organic analysis. I. Identification of drugs by principal components analysis of standardized thin-layer chromatographic data in four eluent systems. J. Chromatogr. **1985**, *350* (1), 151–168.
16. Romano, G.; Caruso, G.; Musumarra, G.; Pavone, D.; Cruciani, G. Qualitative organic analysis. Part 3. Identification of drugs and their metabolites by PCA of standardized TLC data. J. Planar Chromatogr. **1994**, *7* (3), 233–241.
17. Ferenczi-Fodor, K.; Vegh, Z.; Renger, B. Thin-layer chromatography in testing the purity of pharmaceuticals. TrAC, Trends Anal. Chem. **2006**, *25* (8), 778–789.
18. Renger, B. Benchmarking HPLC and HPTLC in pharmaceutical analysis. J. Planar Chromatogr. **1999**, *12* (1), 58–62.
19. Aranda, M.; Morlock, G. Simultaneous determination of riboflavin, pyridoxine, nicotinamide, caffeine and taurine in energy drinks by planar chromatography-multiple detection with confirmation by electrospray ionization mass spectrometry. J. Chromatogr. A **2006**, *1131* (1–2), 253–260.
20. Aranda, M.; Vega, M.; Villegas, R. Routine method for quantification of starch by planar chromatography (HPTLC). J. Planar Chromatogr. **2005**, *18* (104), 285–289.
21. Vega, M.; Jara, E.; Aranda, M. Monitoring the dose of florfenicol in medicated salmon feed by planar chromatography (HPTLC). J. Planar Chromatogr. **2006**, *19*, 204–207.
22. Morlock, G.; Schwack, W. Cromatografía planar-volver al futuro. LabCiencia Not. Tec. Lab. **2008**, *3*, 7–9.
23. Morlock, G.E.; Prabha, S. Analysis and stability of sucralose in a milk-based confection by a simple planar chromatographic method. J. Agric. Food Chem. **2007**, *55* (18), 7217–7223.
24. Morlock, G.E.; Vega-Herrera, M.A. Two new derivatization reagents for planar chromatographic quantification of sucralose in dietetic products. J. Planar Chromatogr. **2007**, *20* (6), 411–417.
25. Morlock, G.; Schwack, W. Coupling of planar chromatography to mass spectrometry. TrAC, Trends Anal. Chem. **2009**, *in submission.*
26. Tames, F.; Watson, I.D.; Morden, W.; Wilson, I.D. Detection and identification of morphine in urine extracts using thin-layer chromatography and tandem mass spectrometry. J. Chromatogr. B **1999**, *729* (1–2), 341–346.

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27. Parent, A.A.; Anderson, T.M.; Michaelis, D.J.; Jiang, G.L.; Savage, P.B.; Linford, M.R. Direct ToF-SIMS analysis of organic halides and amines on TLC plates. Appl. Surf. Sci. **2006**, *252* (19), 6746–6749.
28. Nakamura, K.; Suzuki, Y.; Goto-Inoue, N.; Yoshida-Noro, C.; Suzuki, A. Structural characterization of neutral glycosphingolipids by thin-layer chromatography coupled to matrix-assisted laser desorption/ionization quadrupole ion trap time-of-flight MS/MS. Anal. Chem. **2006**, *78* (16), 5736–5743.
29. Fuchs, B.; Schiller, J.; Suss, R.; Nimptsch, A.; Schurenberg, M.; Suckau, D. Capabilities and disadvantages of combined matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and high-performance thin-layer chromatography (HPTLC): Analysis of egg yolk lipids. J. Planar Chromatogr. **2009**, *22* (1), 35–42.
30. Meisen, I.; Distler, U.; Muthing, J.; Berkenkamp, S.; Dreisewerd, K.; Mathys, W.; Karch, H.; Mormann, M. Direct coupling of high-performance thin-layer chromatography with UV spectroscopy and IR-MALDI orthogonal TOF MS for the analysis of cyanobacterial toxins. Anal. Chem. **2009**, *81* (10), 3858–3866.
31. Van Berkel, G.J.; Tomkins, B.A.; Kertesz, V. Thin-layer chromatography/desorption electrospray ionization mass spectrometry: Investigation of goldenseal alkaloids. Anal. Chem. **2007**, *79* (7), 2778–2789.
32. Lin, S.Y.; Huang, M.Z.; Chang, H.C.; Shiea, J. Using electrospray-assisted laser desorption/ionization mass spectrometry to characterize organic compounds separated on thin-layer chromatography plates. Anal. Chem. **2007**, *79* (22), 8789–8795.
33. Peng, S.; Edler, M.; Ahlmann, N.; Hoffmann, T.; Franzke, J. A new interface to couple thin-layer chromatography with laser desorption/atmospheric pressure chemical ionization mass spectrometry for plate scanning. Rapid Commun. Mass Spectrom. **2005**, *19* (19), 2789–2793.
34. Morlock, G.; Ueda, Y. New coupling of planar chromatography with direct analysis in real time mass spectrometry. J. Chromatogr. A **2007**, *1143* (1–2), 243–251.
35. Haddad, R.; Milagre, H.M.S.; Catharino, R.R.; Eberlin, M.N. Easy ambient sonic-spray ionization mass spectrometry combined with thin-layer chromatography. Anal. Chem. **2008**, *80* (8), 2744–2750.
36. Eberlin, L.S.; Abdelnur, P.V.; Passero, A.; de Sa, G.F.; Daroda, R.J.; de Souza, V.; Eberlin, M.N. Analysis of biodiesel and biodiesel-petrodiesel blends by high performance thin layer chromatography combined with easy ambient sonic-spray ionization mass spectrometry. Analyst **2009**, *134* (8), 1652–1657.
37. Lafleur, J.P.; Salin, E.D. Speciation of chromium by high-performance thin-layer chromatography with direct determination by laser ablation inductively coupled plasma mass spectrometry. Anal. Chem. **2008**, *80* (17), 6821–6823.
38. Resano, M.; Ruiz, E.G.; Mihucz, V.G.; Moricz, A.M.; Zaray, G.; Vanhaecke, F. Rapid screening method for arsenic speciation by combining thin layer chromatography and laser ablation-inductively coupled plasma-dynamic reaction cell-mass spectrometry. J. Anal. Atom. Spectrom. **2007**, *22* (9), 1158–1162.
39. Kertesz, V.; Ford, M.J.; Van Berkel, G.J. Automation of a surface sampling probe/electrospray mass spectrometry system. Anal. Chem. **2005**, *77* (22), 7183–7189.
40. Prosek, M.; Milivojevic, L.; Krizman, M.; Fir, M. On-line TLC-MS. J. Planar Chromatogr. **2004**, *17* (6), 420–423.
41. Luftmann, H. A simple device for the extraction of TLC spots: Direct coupling with an electrospray mass spectrometer. Anal. Bioanal. Chem. **2004**, *378* (4), 964–968.
42. Aranda, M.; Morlock, G. New method for caffeine quantification by planar chromatography coupled with electropray ionization mass spectrometry using stable isotope dilution analysis. Rapid Commun. Mass Spectrom. **2007**, *21* (7), 1297–1303.
43. Aranda, M.; Morlock, G. Simultaneous determination of caffeine, ergotamine and metamizol in solid pharmaceutical formulation by HPTLC/UV/FLD with mass confirmation by online HPTLC/ESI-MS. J. Chromatogr. Sci. **2007**, *45* (5), 251–255.
44. Morlock, G.; Schwack, W. Determination of isopropylthioxanthone (ITX) in milk, yoghurt and fat by HPTLC-FLD, HPTLC-ESI/MS and HPTLC-DART/MS. Anal. Bioanal. Chem. **2006**, *385* (3), 586–595.

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45. Alpmann, A.; Morlock, G. Improvement of online coupling of planar chromatography with electrospray mass spectrometry: Extraction of zones from glass plates. Anal. Bioanal. Chem. **2006**, *386* (5), 1543–1551.
46. Morlock, G. New HPTLC method, with systematic mobile-phase optimization, for determination of six apolar heterocyclic aromatic amines. J. Planar Chromatogr. **2004**, *17* (6), 431–434.
47. International Conference on Harmonisation (ICH). *ICH Harmonised Tripartite Guideline Validation of Analytical Procedures: Text and Methodology Q2 (R1)*; 2005.
48. Morlock, G.E.; Jautz, U. Comparison of two different plunger geometries for HPTLC-MS coupling via an extractor-based interface. J. Planar Chromatogr. **2008**, *21* (5), 367–371.
49. USP/NF. *The United States Pharmacopeia (USP) 25*, The United States Pharmacopeia Convention: Rockville, MD., 2002.
50. Routine, validated method applied in a pharmaceutical company, private communication, 2009.
51. Dytkiewitz, E.; Morlock, G.E. Analytical strategy for rapid identification and quantification of lubricant additives in mineral oil by high-performance thin-layer chromatography with UV absorption and fluorescence detection combined with mass spectrometry and infrared spectroscopy. J. AOAC Intl. **2008**, *91* (5), 1237–1243.

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