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Short communication

Determination of piracetam and its impurities by TLC

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1. Introduction

Piracetam (2-oxo-1-pyrollidine acetamine (I), Fig. 1) which is the prototype of nootropic drugs, is used mostly for the improvement of learning and memory, and in the treatment of myclonus [1,2]. Samples of piracetam may contain some structurally related impurities (Fig. 1) derived from the manufacturing process (2-oxo-pyrollidine (II), 2-oxo-1-pyrollidine methyl acetate (IV) and 2-oxo-1-pyrollidine ethyl acetate (V)) or by degradation (2-oxo-1-pyrollidine acetic acid (III)).

Various analytical methods have been reported for the analysis of piracetam [3–15]. However, to date no chromatographic methods for the simultaneous separation of piracetam from all its known structurally related impurities have been published. This paper describes the development of a simple and reproducible TLC method for the separation and detection of related substances in piracetam. A range of detection reagents were examined for their suitability in providing optimal detection, simultaneously, for all the compounds examined.

2. Experimental

2.1. Reference substances

Piracetam (I) and the related substances (II–V) were supplied by Union Chimique-chemische, Belgium.

2.2. Chemicals and reagents

Ethyl acetate, ethanol, methanol, acetonitrile and chloroform, all HPLC grade and glacial acetic acid (GPR grade) were obtained from Rathburn Chemicals, Scotland. Pentyl acetate

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(SLR grade) was obtained from Fisher Scientific, Loughborough, England. Analar grade 2,6-dichloroquinone-4-chloroimide was obtained from Sigma-Aldrich, St. Louis, USA. All other reagents, obtained from BDH Chemicals, Poole, England, were of Analar quality.

2.3. Standard solutions

2.3.1. Standard solutions of compounds I–V

A stock solution of each compound was prepared in methanol at a concentration of 1.0% w/v. Each standard stock solution was serially diluted to produce reference solutions of concentration 0.010, 0.025, 0.050, 0.100 and 0.500% w/v.

2.4. Piracetam solution (10% w/v)

Piracetam (2.0 g) was accurately weighed and transferred into a 20 ml volumetric flask. Then it was dissolved in, and made up to the mark, with methanol.

2.5. Standard mixture of impurities

Impurities II–V were each weighed accurately (0.05 g) into the same 10 ml volumetric flask. The compounds were dissolved in, and made up to the mark to produce a solution containing 0.5% w/v of each impurity.

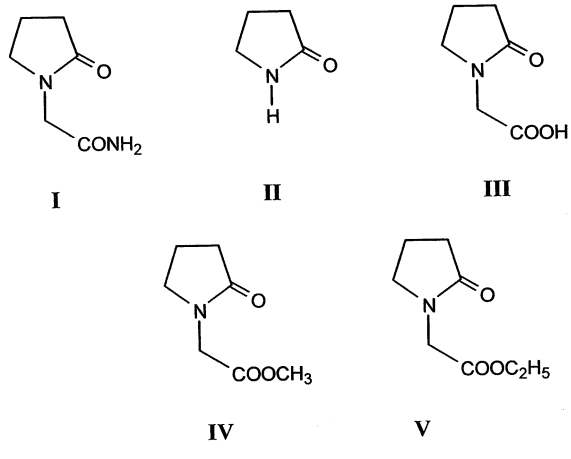

Fig. 1. Chemical structures of piracetam (I) and the related impurities 2-oxo-pyrrolidine (II), 2-oxo-pyrrollidine acetic acid (III), 2-oxo-pyrollidine methyl acetate (IV) and 2-oxo-pyrrollidine ethyl acetate (V).

2.6. Standard mixtures of piracetam and its impurities

Volumes of 1, 2, 3 and 5 ml of the piracetam solution (10% w/v) were added separately to four different 10 ml volumetric flasks each containing 1 ml of the standard mixture of impurities. The flasks were made up to the mark with methanol to produce solutions containing 1, 2, 3 and 4% w/v piracetam in the presence of 0.05% w/v of each impurity.

2.7. Chromatography

Commercial plates coated with Kieselgel $60F_{254}$ (10×10 cm, 0.25 mm layer thickness, HPTLC grade, glass support) and Kieselgel $60F_{254}$ (20×20 cm, 0.25 mm layer thickness, plastic support) were obtained from Merck (Darmstadt, Germany).

For conventional TLC analysis, aliquots (10 μl) of the solutions of the test substances were applied with disposable calibrated micro-capillaries (Camag, Switzerland), starting 3 cm from the side of the Kieselgel $60F_{254}$ plates (20×20 cm). The distance between spots was 1.5 cm. The spots were dried in a gentle stream of hot air.

Development of the plate was carried out using a normal vertical development tank at ambient temperature, with 45 min tank saturation. A quaternary solvent system composed of pentyl acetate–ethyl acetate–ethanol–glacial acetic acid (10:10:9:1 v/v/v/v) was used.

For TLC with UV densitometry analysis, solutions of the test substances were applied to Kieselgel $60F_{254}$ HPTLC plates (10×10 cm) using a Camag-Nanomat III automatic spotter (Camag, Switzerland) equipped with calibrated 1 μl glass capillaries. Development of the plates was carried out using a horizontal development chamber (10×20 cm, Camag, Switzerland) at ambient temperature, with 15 min tank saturation. A Shimadzu CS-9000 dual wavelength flying spot scanner was used for densitometric evaluation of the plates at the following settings; mode: ab-

Table 1
TLC detection reagents for piracetam (I) and its impurities (II–V)

Detection method	Visibility
UV light (254 nm)	(+)
Ninhydrin [18]	(−)
Vanillin [17]	(−)
Iodine vapour [17]	(+)
Iodine-starch solution [17]	(++)
Iodine chloroformic solution [17]	(++)
Iodine chloroformic starch solution [17]	(++)
2,4 dinitrophenyl hydrazine [19]	(−)
Ehrlich's reagent [17]	(−)
Hydroxylamine-iron (II) chloride [18]	(−)
Dragendorf reagent [20]	(−)
Gibb's reagent [19]	(++)
Gibb's reagent–ammonia vapour [19]	(+++)
Potassium permanganate–acetic acid [17]	(−)

Table 2
TLC data for piracetam (I) and its impurities (II–V)

Compound	R_F (mean[RSD], $n = 5$)
I	0.21 (2.1%)
II	0.34 (2.6%)
III	0.04 (12.4%)
IV	0.47 (3.5%)
V	0.54 (1.7%)

sorbance/reflectance; sample wavelength: 210 nm; reference wavelength: 230 nm; zero set mode: at start; beam size: 0.4 × 0.4mm: δy: 0.04 mm; beam swing width: 4 mm; scan mode: zigzag; minimum spot-width detection: 0.5 mm.

3. Results and discussion

Piracetam and its structurally related impurities (II–V) are simple molecules containing a basic 2-oxo-pyrrolidine ring (a cyclic amide) which is not very reactive. Consequently not many reagents are suitable for detecting these compounds. Reagents such as iodine vapour and chlor-toluidine have been used to detect piracetam [10], but the former lacks sensitivity and the latter is carcinogenic. Therefore, a range of other reagents were examined for their suitability to detect piracetam and its impurities by producing a coloured product (Table 1). Although the use of Gibb's reagent (1% w/v solution of 2,6-dichloroquinine-4-chloroimide in absolute ethanol) for detecting piracetam has not been reported in the literature, it was shown to be the most suitable method for the detection of piracetam (I) by comparison with the other reagents examined. Piracetam and its impurities upon spraying with Gibb's reagent produced permanent brown spots which were enhanced after subsequent exposure to ammonia vapour.

To date there have been no reports in the literature for the simultaneous analysis of piracetam and its impurities. Using data on solvent elution strengths [16,17], different solvents and combinations thereof were examined for separating piracetam and its impurities from each other on Kieselgel $60F_{254}$ plates using Gibb's reagent–ammonia vapour. The concentration of each component in the reference solution was 0.5% w/v in methanol. The critical separation was found to be between the two piracetam esters (IV and V), which differ only by one methylene group (Fig. 1). This small structural difference was exploited for separation by incorporating mixtures of esters in the solvent system. A suitable solvent system was optimised to the following conditions: pentyl acetate–ethyl acetate–ethanol–glacial acetic acid (10:10:9:1). The results obtained using this solvent system, Kieselgel $60F_{254}$ plates and Gibb's reagent are shown in Table 2. The reported values are the means of five replicate experiments.

The detection limits of piracetam and each of the impurities were determined by the application of aliquots (10 μl) of the reference solutions (I), II–V, in the concentration range of 0.01–0.1% w/v, followed by detection with Gibb's reagent–ammonia vapour after TLC. The results demonstrated that piracetam (I) and impurities II–V possess similar responses and that the detection limit in each case was approximately 2.5 μg (equivalent to 0.025% w/v) on plate. Using the standard mixtures of piracetam and its impurities, the impurities were detectable in solutions containing 2% w/v of piracetam and 0.05% w/v of each, corresponding to a limiting concentration of 2.5%, making the method suitable for samples

containing piracetam and any of the impurities at a ratio of 40:1 (w/w). Whilst compounds IV and V were detectable in solutions containing 3% w/v of piracetam and 0.05% w/v of each impurity, corresponding to a limiting concentration of 1.7% the high concentration of piracetam masked the detection of compounds II and III.

The separation of conventional Kieselgel $60F_{254}$ plates (20 × 20 cm, 0.25 mm layer thickness, plastic support) was transferred to Kieselgel $60F_{254}$ HPTLC plates (10 × 10 cm, 0.25 mm layer thickness, glass support) using the same solvent system. Since piracetam and its related substances possess insignificant UV-absorption, a detection wavelength of approximately 210 nm was necessary for detection using densitometric scanning. The interference from background noise, which is significant below 230 nm, was compensated for by operating the densitometer in dual wavelength mode with a sample wavelength of 210 nm and a reference wavelength of 230 nm. The instrument stability was assessed by determining the precision of peak area values obtained for the successive scans ($n = 5$) of the same lane containing a spot of piracetam. The precision (relative standard deviation) was $< 1.0\%$ over the experimental period. The limit of detection (3 × signal to noise ratio) of piracetam using UV densitometry was determined as 1.5 μg on plate. A densitogram of the separation of piracetam (I) and its impurities at the same concentration on Kieselgel $60F_{254}$ HPTLC plates is shown in Fig. 2. The R_F values obtained for I–V were 0.40, 0.60, 0.25, 0.78 and 0.86, respectively, demonstrating reasonable separation.

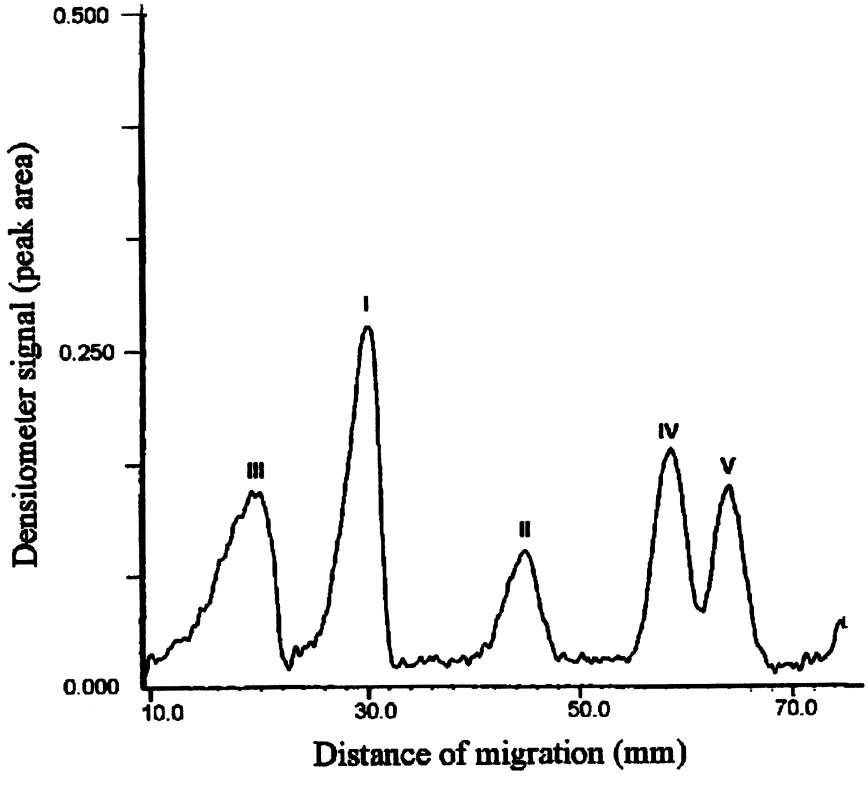

Fig. 2. Densitogram of the chromatographic separation of piracetam (I) and its related substances (II–V) on HPTLC grade Kieselgel $60F_{254}$ plates with a quaternary mobile phase of pentyl acetate–ethyl acetate–ethanol–glacial acetic acid (10:10:9:1, v/v/v/v). Detection was at sample and reference wavelengths of 210 and 230 nm, respectively.

4. Conclusion

A simple and reproducible TLC method has been described for the separation of piracetam from its manufacturing and degradation impurities. All the analytes were separated from each other on both conventional and HPTLC Kieselgel $60F_{254}$ plates with a quaternary mobile phase of pentyl acetate–ethyl acetate–ethanol–glacial acetic acid (10:10:9:1, v/v/v/v). Detection of piracetam and its impurities was accomplished using Gibb's reagent–ammonia vapour or dual-wavelength UV scanning densitometry (sample: 210 nm; reference: 230 nm). The methods are thought to be suitable for the routine control of the four impurities because all substances exhibited similar detector responses.

Acknowledgements

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References

[1] USP DI, Drug Information for the Health Care Professional, vol. 1, United States Pharmacopoeial Convention, Inc., USA, 1995, p. 2991.
[2] C.R. Wilsher, Int. J. Psychophysiol. 4 (1996) 29–39.
[3] A.H. Gouliaev, J.B. Monster, M. Vedso, A. Senning, Org. Preparations Procedures Int. 27 (1995) 273–303.
[4] T. Alebic-Kolbach, S. Hirls-Starcevic, J. Chromatogr. 526 (1990) 556–561.
[5] C. Altomare, S. Callamare, A. Crotty, G. Cassini, E. Gavuzzo, F. Mazza, J. Med. Chem. 38 (1995) 170–179.
[6] W. Deberdt, Life Sci. 55 (1994) 2057–2066.
[7] R.M. Nalbaldian, M.F. Kubicek, W.J. O'Brien, B. Nichols, L. Henry, G.A. Williams, A.I. Goldman, D. Adams, C.M. Teng, Clin. Chem. 29 (1983) 664–666.
[8] L. Gugliardi, D. de Orsi, G. Cavazzutti, D. Tonelli, S. Zapolli, Anal. Lett. 27 (1994) 879–885.
[9] E. Pawelczyk, B. Smilowski, Acta Polon Pharm. 41 (1984) 351–358.
[10] H.D. Dell, J. Flieder, H. Jacobi, R. Kamp, Frezenius Z. Anal. Chem. 304 (1980) 407–411.
[11] W. Rieck, D. Platt, J. Chromatogr. B 232 (1982) 203–206.
[12] C. Hesse, M. Shulz, Chromatographia 12 (1979) 12–13.
[13] K. Louchachi, M. Told, P. Bonnardel, O. Petitjean, J. Chromatogr. B 633 (1995) 385–389.
[14] H. Mascher, C. Kikuta, J. Pharm. Biomed. Anal. 7 (1989) 913–916.
[15] T. Daldrup, F. Susanto, P. Michalke, Frezenius Z. Anal. Chem. 308 (1981) 413–427.
[16] J.C. Touchstone, D. Rogers, Thin Layer Chromatography Quantitative Environmental and Clinical Applications, John Wiley & Sons, New York, 1980.
[17] E. Stahl, Thin Layer Chromatography, Springer-Verlag, Academic Press Inc., Publishers, New York, 1965.
[18] E. Merck, Dyeing Reagents for Thin Layer and Paper Chromatography, E. Merck Publishers, Germany, 1974.
[19] Macherey-Nagel-Duren, Thin Layer Chromatography, Macherey-Nagel GmbH & Co, Germany, 1989.
[20] A.C. Moffat, Clarke's Isolation and Identification of Drugs, The Pharmaceutical Press, London, 1986.