J. Serb. Chem. Soc. 71 (6) 669–676 (2006) UDC 547.583.2+543.544:615.453.6+661.187.7
JSCS–3460 *Original scientific paper*

Determination of saccharin in pharmaceuticals by high performance thin layer chromatography

MIRA ČAKAR* and GORDANA POPOVIĆ#

Faculty of Pharmacy, University of Belgrade, P. O. Box 146, Vojvode Stepe 450, 11000 Belgrade, Serbia (e-mail: mcakar@pharmacy.bg.ac.yu)

(Received 24 May, revised 15 November 2005)

Abstract: A simple, accurate and selective high performance thin layer chromatographic method for the determination of saccharin in pharmaceuticals has been developed. The chromatography was performed on silica-gel $60F_{254}$ plates with ethyl acetate–carbon tetrachloride–acetic acid (3 + 4 + 0.5 v/v/v) as the mobile phase. The chromatographic zones corresponding to the saccharin spots were scanned in the reflectance/absorbance mode at λ = 230 mm. For the standard curves, two series of saccharin sodium salt solutions were prepared: in methanol (solvent 1) and in ethyl acetate–acetic acid (9:1, v/v) mixture (solvent 2). A linear calibration relationship was observed within the concentration range from 300 – 1200 ng saccharin sodium salt per spot, correlation coefficients being 0.998 (solvent 1) and 0.995 (solvent 2). The relationship between the peak area and the amount of saccharin sodium salt was evaluated by linear regression analysis. The limits of detection and quantification of saccharin sodium salt were 35 ng and 110 ng per spot (solvent 1), respectively, and 45 ng and 150 ng per spot (solvent 2), respectively. Mean recovery values of 103.5 % (solvent 1) and 102.3 % (solvent 2), and RSD values of 4.42 % (solvent 1) and 2.53 % (solvent 2) were obtained. The proposed method was applied for saccharin determination in two pharmaceutical preparations, effervescent tablets and a carbomer-based gel.

Keywords: saccharin, HPTLC determination, effervescent tablets, carbomer-based gel.

INTRODUCTION

Saccharin (1,2-benzisothiazolin-3-one-1,1-dioxide) and its salts are intense sweeteners, being in dilute aqueous solutions about 300–500 times sweeter than sucrose.[1] Saccharin is not readily soluble in water, but its commercially available sodium salt, used as a non-nutritive sweetener, is freely soluble in water. Saccharin is excreted unchanged by the body within 24 to 48 h. There have been sparse reports on hypersensitivity and photosensitivity reactions to saccharin. Despite the

* Author for correspondence.

Serbian Chemical Society active member.

doi: 10.2298/JSC0606669Č

fact that saccharin causes urinary bladder cancer in laboratory rats, its ban was rescinded after a public outcry. In 1984, the World Health Organization suggested an intake limit of 2.5 mg/day/ kg b.w.

Hitherto, saccharin has been determined in pharmaceuticals by spectroscopic,[2–15] electrochemical,[16,17] titrimetric[18] and different chromatographic techniques, such as gas chromatography,[10,19] high performance liquid chromatography[20–26] and ion chromatography.[27] Thin layer chromatography (TLC) was applied for the separation and identification of saccharin,[10,28] but only a single paper[29] was devoted to a TLC method for the determination of saccharin (limit of detection 4.0 μg/spot). This method involved the use of Al_2O_3/polyamide layers and toluene–acetic acid–formic acid (5 + 4 + 2, v/v/v) mixture as the mobile phase. A mixture of bromocresol green–bromophenol blue with $KMnO_4$ solution was employed as the chromogenic spray reagent. Titrimetry,[30–33] liquid chromatography[31] and spectrophotometry[31] are the official methods for saccharin determination issued by different pharmacopoeias.

Advancement in instrumentation as a result of the development of microcomputer techniques, as well as the modifications of old and the discovery of new chromatographic stationary phases has resulted in the increasing application of thin-layer chromatography in quantitative analysis. The possibility for the separation of components present in a mixture and the simultaneous handling of a large number of samples has led to an extensive use of TLC for the analysis of pharmaceutical preparations and food products.[34–40]

In the present study, a simple, reliable and selective HPTLC method for the determination of saccharin ws developed. The method was applied for saccharin determination in two preparations – effervescent tablets and a carbomer-based gel, each requiring a different approach for the preparation of the to be analyzed saccharin solutions. Namely, it was possible to directly determine saccharin in effervescent tablets, while its determination in a carbomer-based gel necessitated the prior decomposition of the gel structure and extraction.

EXPERIMENTAL

Reagents and apparatus

Saccharin sodium salt and all other reagents were Merck (Darmstadt, Germany) products of analytical grade purity. In the present work, the investigated formulations contained saccharin as its sodium salt: Kalcijum® effervescent tablets (label claim of saccharin sodium salt and calcium 5 mg and 200 mg per tablet, respectively) produced by Hemofarm Pharmaceutical Works (Vršac, Serbia) and Daktanol® gel (labeled claim of miconazole 20 mg per g gel; no declared amout of saccharin sodium salt), a product of Galenika Pharmaceutical Works (Belgrade, Serbia).

For the chromatographic investigations, a TLC Scanner II with a computer system and Cats Software V. 3.15 (Camag, Switzerland) were used. A Nanomat II (Camag) was employed as the application device. Chromatoplates 20×10 cm Silica gel $60F_{254}$ were purchased from Merck (Germany). The chromatograms were developed in a twin-through chamber (Camag).

Procedure

Standard solutions. Two series of standard solutions for the calibration curves were prepared, one of them serving for saccharin sodium salt determination in effervescent tablets and the second one for its determination in the medicinal gel. For these purposes, saccharin sodium, salt solutions (0.10, 0.15, 0.20, 0.25, 0.30, 0.35 and 0.4 mg/ml) were prepared in methanol (effervescent tablets) and ethyl acetate–acetic acid (9:1, v/v) mixture (medicinal gel).

Sample solutions. Effervescent tablets. Twenty tablets were pulverized and the amount corresponding to the average mass of a single tablet was mixed with 20 ml methanol. The mixture was treated for 10 min in an ultrasonic bath and filtered (blue strip filter paper). The filtrate (3 µl) was applied to the chromatographic plate.

Medicinal gel. A mixture of 2.5 g of the gel and 2.5 g of $KHSO_4$ in a conical flask with a ground-glass stopper was stirred on a magnetic stirrer for 15 min at 50 °C. Subsequently, 10 g anhydrous Na_2SO_4 were added and the stirring was continued with a glass rod for 15 min at ambient temperature. Then, 10 ml ethyl acetate was introduced and the mixture shaken for a further 15 min. The mixture was filtered and the filtrate (3.0 µl) was applied to the chromatographic plate.

Chromatography. Standard and sample solutions (3.0 µl) were applied to the HPTLC plates and the chromatograms were developed in a twin-through chamber using ethyl acetate–carbon tetrachloride–acetic acid (3 + 4 + 0.5, v/v/v) as the mobile phase (without saturation). Ascending chromatography was performed to a distance of 95 mm and a developing time of 33 – 35 min. The plates were air-dried and the spots were scanned in the linear reflectance/absorbance mode at 230 nm. Peak areas were used for quantification.

RESULTS AND DISCUSSION

Chemically, saccharin represents an acid, slightly soluble in water. The preparations examined throughout the present study, *i.e.*, effervescent tablets and a carbomer-based gel, contained saccharin sodium salt which is freely soluble in water and alcohol. For the analyses of the effervescent tablets, methanol was used as the solvent because saccharin sodium salt is readily soluble in methanol while the other components of the tablets are insoluble. The other examined pharmaceutical preparation Daktanol® gel contains a carbomer serving as an emulsion stabilizer and viscosity adjuster. The examined gel is soluble in methanol, but when its methanol solution was directly subjected to chromatographic analysis, a smeared spot was obtained. For this reason, it was necessary to destroy the network structure of the carbomer and extract the saccharin with ethyl acetate. Carbomer-based gels contain a high percentage of water and decomposition of their structure can be achieved by the addition of electrolytes. In the case of Daktanol® gel, potassium hydrogensulphate and anhydrous sodium sulphate were used for this purpose. In addition to its participation in the decomposition of the gel structure, potassium hydrogensulphate provided an acidic medium, while anhydrous sodium sulphate served as a water adsorbent.

Representative chromatograms of standard saccharin solutions and the examined preparations obtained by HPTLC are shown in Fig. 1. Ethyl acetate–carbon tetrachloride–acetic acid mixture (3 + 4 + 0.5, v/v/v) as the mobile phase provided a well-defined saccharin peak. Detection was performed at 230 nm. This wavelength was chosen on the basis of the UV spectrum of saccharin obtained in-situ

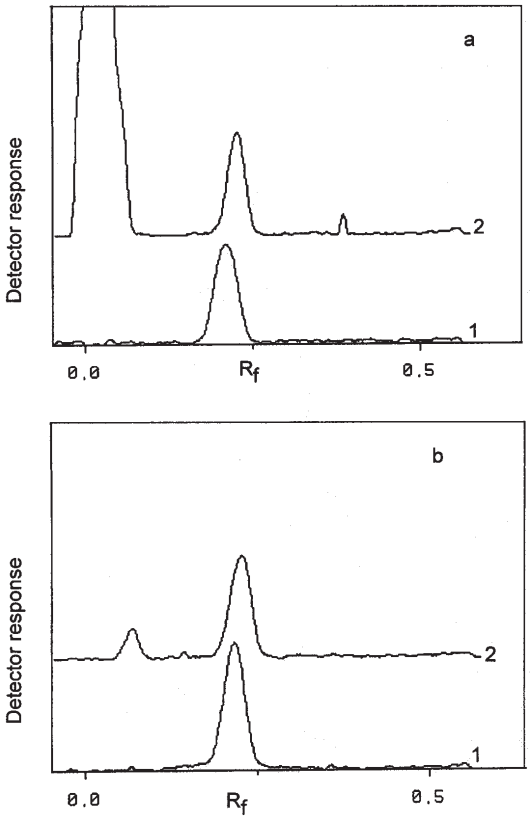

Fig. 1. Chromatograms of: a) saccharin standard (1) and Kalcijum® effervescent tablets (2); b) saccharin standard (1) and Daktanol® gel (2); λ = 230 nm.

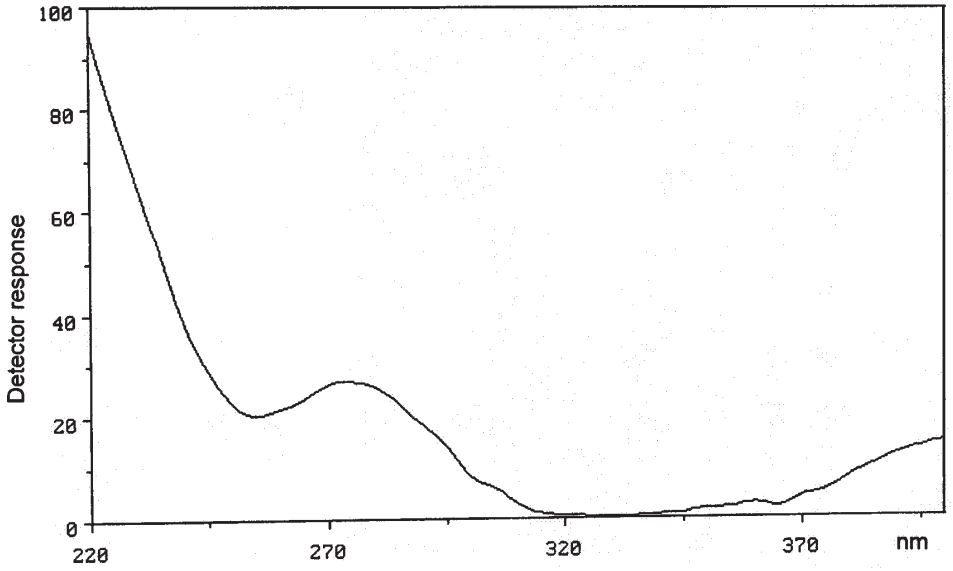

Fig. 2. In-situ UV spectrum of saccharin obtained from the chromatographic band.

from the chromatographic band (Fig. 2). From Fig. 2, it can be seen that the wavelength of 275 nm represents an absorption maximum of saccharin, while the absorption was even higher at wavelengths below 245 nm, increasing abruptly with decreasing wavelength. For this reason, the determinations in the present work were performed at 230 nm with the aim of achieving a higher sensitivity. In spite of a higher sensitivity, the determinations at wavelengths below 230 nm gave results with relative standard deviations above 5 %.

TABLE I. The effect of the solvents on the regression data for the calibration curves of saccharin sodium salt in the concentrationrange from 300 – 1200 ng/spot (n = 7)

Parameter	Solvent used to prepare the standard solutions	
	Methanol	Ethyl acetate–acetic acid (9:1, v/v)
Slope	1.421	1.168
Standard deviation of the slope	0.034	0.054
Intercept	–49.88	–67.83
Standard deviation of the intercept	29.18	45.83
Correlation coefficient	0.998	0.995

The calibration curve, obtained within the range of 300 – 1200 ng saccharin sodium salt per spot, was characterized by a linear relationship between the peak area and the content of saccharin sodium salt. Bearning in mind that the peak shape was influenced not only by the stationary and mobile phase, but also by the solvent, standard solutions used for the calibration curves were prepared in two solvents, *i.e.*, in methanol for the analyses of the effervescent tablets and in an ethyl acetate – acetic acid mixture (9:1, v/v) for the analyses of the gel. The regression analysis data for the calibration curves are listed in Table I. A high correlation for both calibration curves can be seen. It is also obvious that a higher sensitivity was achieved when methanol was used as the solvent. This could be explained in terms of different forms of saccharin being present in the solvents used (salt in methanol and acid in the ethyl acetate–acetic acid mixture). This led to the differences in the values of the limits of detection and the experimentally obtained quantification of saccharin sodium salt, on the basis of signal-to-noise ratios of 3:1 and 10:1, respectively (Table II).

The accuracy of the developed method was established by the method of standard addition. The precision data were obtained from six-replicate analyses in the accuracy study. The recovery and RSD values listed in Table III demonstrate a satisfactory accuracy and precision of the method.

TABLE II. The effect of the solvents on the limit of detection (LOD) and quantification (LOQ) of saccharin sodium salt

Solvent	LOD (ng/spot)	LOQ (ng/spot)
Methanol	35	110
Ethyl acetate–acetic acid (9:1, v/v)	45	150

TABLE III. The effect of the solvents on the accuracy and precision of the method (n = 6)

Solvent	Amount of saccharin sodium salt (ng/spot)		RSD/%	Recovery %
	Taken	Found*		
Methanol	750.0	776.2 ± 34.3	4.42	103.5
Ethyl acetate–glacial acetic acid (9:1,v/v)	750.0	767.3 ± 19.4	2.53	102.3

*Mean ± standard deviation

TABLE IV. Content of saccharin sodium salt in Kalcijum® effervescent tablets and Daktanol® gel (n = 6)

Pharmaceutical	Found mg	RSD/%	Percentage of the label claim
Kalcijum® effervescent tablets (label claim: 5.0 mg saccharin sodium salt tablet)	5.08[a]	1.67	101.5
Daktanol® gel (no label claim for saccharin sodium salt content)	1.03[b]	4.58	–

[a]mg/tablet; [b]mg/g gel

The proposed method was used for the determination of saccharin in Kalcijum® effervescent tablets and Daktanol® gel. The obtained results are summarized in Table IV.

CONCLUSION

The method proposed for the determination of saccharin in effervescent tablets and a carbomer-based gel is simple, reliable and highly selective. Also, eighteen samples per plate can be analysed simultaneously. In relation to a previously described TLC approach, the method proposed herein is more sensitive and enables a direct densitometric determination of sacharin without the use of chromogenic reagents.

Acknowledgements: This work was supported by the Ministry for Science and Environmental Protection of Serbia.

ИЗВОД

ОДРЕЂИВАЊЕ САХАРИНА У ФАРМАЦЕУТСКИМ ПРЕПАРАТИМА ПРИМЕНОМ ВИСОКО ЕФИКАСНЕ ТАНКОСЛОЈНЕ ХРОМАТОГРАФИЈЕ

МИРА ЧАКАР и ГОРДАНА ПОПОВИЋ

Фармацеутски факултет, Универзитет у Београду, п. пр. 146, Војводе Степе 450, 11000 Београд

Предложена је једноставна и селективна метода за одређивање сахарина у фармацеутским препаратима применом високо ефикасне танкослојне хроматографије. Одређивања су вршена на силика-гел плочама $60F_{254}$ користећи као мобилну фазу смешу етилацетата, угљентетрахлорида и сирћетне киселине (3 + 4 + 0,5, v/v/v). Припремљене су две серије стандардних раствора натријумове соли сахарина: у метанолу (растварач 1) и смеши етилацетат-сирћетна киселина (9:1, v/v) (растварач 2). Калибрационе криве сним-

ђене су на таласној дужини од 230 nm у опсегу нанетих количина натријумове соли сахарина (300 – 1200 ng), са коефицијентима корелације 0,998 (растварач 1) и 0,995 (растварач 2). У испитиваном опсегу утврђена је линеарна зависност између површине пикова и количине натријумове соли сахарина. Лимит детекције и лимит квантификације сахарина износили су редом 35 ng и 110 ng (растварач 1) и 45 ng и 150 ng (растварач 2). Добијене су следеће вредности за „recovery" и релативну стандардну девијацију: 103,5 % и 4,42 % (растварач 1) и 102,3 % и 2,53 % (растварач 2). Предложена метода је примењена за одређивање сахарина у шумећим таблетама Калцијум® и Дактанол® гелу.

(Примљено 24. маја, ревидирано 15. новембра 2005)

REFERENCES

1. Martindale, *The Extra Pharmacopoeia,* 33rd Edition, The Pharmaceutical Press, London, 2002, p. 1375
2. C. D. Vianna-Soares, J. L. S. Martins, *Rev. Brasil. Cienc. Farm.* **38** (2002) 471
3. O. Agrawal, J. V. Das, V. K. Gupta,*J. Indian Chem. Soc.* **76** (1999) 61
4. M. C. Yebra, M. Gallego, M. Valcarcel, *Anal. Chim. Acta* **308** (1995) 275
5. I. Lopez Garcia, C. Sanchez-Pedreno, F. Conteras Contreras, *Anal. Cienc.* **45** (1986) 25
6. S. Di Pasquale, F. Corigliano, *Ann. Fac. Econ. Commer.* **6** (1968) 149
7. T. Gowda, M. Gowda, K. S. Rangappa, *Anal. Lett.* **17** (1984) 149
8. M. A. Korany, A. M. Wahbi, M. A. Elsayed, S. Mandour, *Farmaco* **39** (1984) 243
9. P. G. Ramappa, A. N. Nayak, *Analyst* **108** (1983) 966
10. J. Alary, J. Arnaud, A. Villet, *Bull. Trav. Soc. Pharm. Lyon* **25** (1981) 58
11. A. A. Al-Badr, M. S. El-Din Rashed, S. E. Ibrahim, *Pharmazie* **37** (1982) 305
12. Y. A. Beltagy, *Pharmakotherapie und Laboratoriumsdiagnostic* **116** (1977) 925
13. N. Oi, E. Inaba, *Yakugaku Zasshi* **87** (1967) 640
14. I. K. Shih, F. W. Teare, *Can. J. Pharm. Sci.* **1** (1966) 35
15. Y. Nagase, S. Baba, M. Suzuki, *Yakugaku Zasshi* **79** (1959) 705
16. R. K. Chernova, O. V. Barinova, E. G. Kulapina, E. A. Materova, *J. Anal. Chem*. **50** (1995) 711
17. Z. Nasierowska, *Farm. Pol.* **31** (1975) 975
18. T. Kashima, *Yakugaku Zasshi* **74** (1954) 101
19. E. M. Ratchik, V. Viswanathan, *J. Pharm. Sci.* **64** (1975) 133
20. A. M. Di Pietra, V. Cavrini, D. Bonazzi, L. Benfenati, *Chromatographia* **30** (1990) 215
21. O. Agostini, A. Chiari, D. Ciofi Baffoni, *Boll. Chim. Farm.* **121** (1982) 612
22. P. J. Palermo, P. S. Tsai, *J. Pharm. Sci.* **68** (1979) 878
23. L. Ciraolo, R. Calapaj, M. T. Clasadonte, *Rass. Chim.* **29** (1977) 181
24. M. Tenenbaum, G. E. Martin, *J. AOAC* **60** (1977) 1321
25. L. Amoretti, E. Gaetani, C. F. Laureti, *Acta Natur.* **12** (1976) 195
26. C. Nsengiyumva, J. O. De Beer, W. Van de Wauw, A. J. Vletinck, F. Parmentier, *Chromatographia* **44** (1997) 634
27. Q. C. Chen, J. Wang, *J. Chromatogr. A* **937** (2001) 57
28. Z. Nasierowska, *Farm. Pol.* **31** (1975) 940
29. M. I. La Rotonda, L. Ferrara, *Boll. Soc. Ital. Biol. Sperim.* **51** (1975) 690
30. *British Pharmacopoeia* (1998) p. 1147
31. *The United States Pharmacopoeia* (1998) p. 1393
32. *European Pharmacopoeia 5.0* (2005) p. 2387
33. *Pharmacopoeia Jugoslavica 2000* (2001) p. 921
34. D. Agbaba, K. Vučićević, V. Marinković, *Chromatographia* **60** (2004) 223
35. N. P. Singh, A. P. Gupta, A. K. Sinha, P. S. Ahuja, *J. Chromatogr. A* **1077** (2005) 202
36. J. Bouligand, T. Storme, I. Laville, L. Mercier, O. Oberlin, G. Vassal, P. Bourget, *J. Pharm. Biomed. Anal.* **38** (2005) 180

37. O. Grozdanović, D. Antić, D. Agbaba, *J. Sep. Sci.* **28** (2005) 575
38. S. N. Meyyananthan, B. Suresh, *J. Chromatogr. Sci.* **43** (2005) 73
39. E. Gravel, P. Bourget, L. Mercier, A. Paci, *J. Pharm. Biomed. Anal.* **39** (2005) 581
40. I. Vovk, B. Simonovska, H. Vuorela, *J. Chromatogr. A* **1077** (2005) 188.