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Spot-test identification and rapid quantitative sequential analysis of dipyrone
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CONCLUSION

From the results obtained in the current study, the relative proportion of these flavonoids was reduced by maceration conventional technique, while microwave and ultrasonic techniques in combination with 70% ethanol solvent were the most efficient. It may suggest that microwave and ultrasonic methods using 70% ethanol are suitable for fast extraction of flavonoids in a simple way, also considering extraction yield and extraction time. These methods also permitted the acquisition of flavonoids from reduced raw plant material.

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Resumo: O presente estudo teve como objetivo verificar a melhor metodologia de extração para rápida e eficiente obtenção de flavonóides a partir de *Alpinia zerumbet*. Folhas secas foram extraídas com água destilada e etanol 70%, utilizando as metodologias de extração: maceração sob agitação, ultrassom, microondas e agitador. Para verificação dos flavonóides rutina e kaempferol-3-*O*-glicuronídeo foram utilizadas as técnicas de CCD e CLAE em fase reversa. O solvente etanol 70% foi mais eficiente como extrator. Para as metodologias ultrassom, microondas e agitador, não houve variação significativa para o rendimento utilizando etanol 70% (11 a 14%). A concentração relativa de rutina e kaempferol-3-*O*-glicuronídeo, respectivamente, foi maior pelos métodos de extração por ultrassom (1,5 e 5,62 mg g^{-1} folha seca) e microondas (1,0 e 6,64 mg g^{-1} folha seca), utilizando etanol 70%. Procedimentos rápidos e simplificados de extração otimizam o trabalho fitoquímico e a obtenção de metabólitos secundários.

Palavras-chave: cromatografia líquida de alta eficiência, maceração, microondas, ultrassom, Zingiberaceae

BIBLIOGRAPHY

[1] C. P. Victório, R. M. Kuster, C. L. S. Lage, Braz. J. Med. Plant 11 (2009) 147.
[2] R. Soares de Moura, A. F. Emiliano, L. C. R. Carvalho, M. A. V. Souza, D. C. J. Guedes, Cardiovas. Pharmacol. 46 (2005) 288.
[3] L. A. M. A. da Costa, S. M. Morais, M. C. B. R. Dantas, R. A. C. M. Lobo, M. C. Fonteles, Rev. Bras. Farmacol. 79 (1998) 96.
[4] M. A. Mpalantinos, R. Soares de Moura, J. P. Parente, R. M. Kuster, Phytother. 12 (1998) 442.
[5] B. H. Havsteen, Pharmacol. Therapeutics 96 (2002) 67.
[6] C. P.Victório, S. G. Leitão, C. L. S. Lage, J. Ess. Oil Res. 22 (2010) 52.
[7] X. Pan, G. Niu, H. Liu, J. Chromatogr A 922 (2001) 371.
[8] C. P.Victório, C.A.S. Riehl, C. L. S. Lage. J. Ess. Oil-Bear. Plants 12 (2009) 137.
[9] X. Pan, G. Niu, H. Liu, Chem. Eng. Process. 42 (2003) 129.
[10] D. P. Fulzele, R. K. J. Satdive, J. Chromatogr. A 1063 (2005) 9.
[11] A. H. Goli, M. Barzegar, M. A. Sahari, Food Chem. 92 (2004) 521.
[12] N.Turkmen, F. Sari, Y. S. Velioglu, Food Chem. 99 (2006) 835.
[13] R. M. S. Celeghini, J. H. Y. Vilegas, F. M. Lanças, J. Braz. Chem. Soc. 12 (2001) 706.
[14] Y. Yang, F. Zhang, Ultras. Sonochem. 15 (2008) 308.
[15] A. A. Craveiro, F. J. A. Matos, J. W. Alencar, M. M. Pumel, Flav. Frag. J. 4 (1989) 43.
[16] K. Ganzer, I. Szinai, A. Salgo, J. Chromatogr. 520 (1990) 257.
[17] E. Rijke, P. Out, W. M. A. Niessen, F. Ariese, C. Gooijer, U. A. Th. Brinkman, J. Chromatogr., A 112 (2006) 31.
[18] B. H. Oliveira, T. Nakashima, J. D. S. Filho, F. L. Frehse, J. Braz. Chem. Soc. 12 (2001) 243.
[19] L. Tao, Z. T. Wang, E. Y. Zhu, Y. H. Lu, D. Z. Wei, South African J. Bot. 72 (2006) 163.
[20] C. A. Williams, J. B. Harborne, Biochem. Syst. Ecol. 5 (1977) 221.

to classical COX-inhibitors, such as aspirin-like drugs, dipyrone has no anti-inflammatory effect and a low gastrointestinal toxicity, indicating a different mode of action. The authors suggested that the pharmacologically active metabolites of dipyrone inhibit COX activity by sequestering radicals that initiate the catalytic activity of this enzyme or through the reduction of the oxidized states of the COX protein [6]. After oral intake, it is spontaneously hydrolyzed in the gastric fluid to its main metabolite, 4-methylaminoantipyrine (4-MAA), which is rapidly and nearly completely absorbed. 4-MAA is then converted to variety of metabolites by various enzymatic reactions. The effect of dipyrone occurs approximately fifteen minutes after oral administration. The biotransformation takes place at the hepatic level, the duration of this effect is approximately 4-6 hours, and its elimination occurs at the renal level [7].

Recently, additional beneficial effects of dipyrone, such as vascular smooth muscle relaxation, and as antiapoptotics and anticolvulsants, have been reported and have increased the interest in dipyrone [3].

A great problem related with pharmaceutical products around the world is falsification. These incidents probably occur more frequently in developing and poor countries but certainly Europe and United States are not completely free of them. In Brazil the problem was denounced some years ago [8,9] and involved antibiotics, contraceptives, cancer medicines and also common analgesics and antipyretics like dipyrone and aspirin. There is suspicion that such anomalies still remain. From these considerations, it is clearly apparent that it is very important to develop quick, simple, reliable and low cost analytical procedures that could be used routinely for screening examinations to detect possible falsifications.

Some methods have been developed for dipyrone determination, such as titrimetry [1], HPLC [10], spectrophotometry [11-15], potentiometry [16], amperometry [17,18], turbidimetry [19], voltammetry [20], and reflectometry [21]. The iodometric titration of dipyrone is recommended by the Pharmacopoeia [1] but this procedure is very slow and laborious, thus less applicable to large-scale analysis.

The aim of the present work is to develop a simple and reliable spectrophotometric method for the qualitative spot-test [22] associated with a rapid quantitative sequential analysis of dipyrone in pharmaceutical preparations. A similar qualitative procedure has been reported [23] but in the present work the sequential quantitative analysis using iron III as catalyst was developed. The method is based on the selective oxidation of dipyrone, a characteristic reaction of a pyrazolone, in the presence of concentrated sulfuric acid, splitting off formaldehyde which reacts with chromotropic acid producing a reddish-violet compound [11]. The sulfuric acid addition promotes the oxidation of dipyrone and guarantees the temperature necessary for the reaction, to occur, as it interacts with the small quantity of water intentionally added in order to take advantage of the highly exothermic process of the hydration of this acid. The formation of the reddish-violet compound identifies the dipyrone. In case of positive qualitative result the quantitative analysis can be sequentialy performed.

Experimental

Reagents

All chemicals were of analytical grade and were used without further purification. Concentrated sulfuric acid (96%) was obtained from Synth®. Chromotropic acid (disodium salt, dihydrate, $C_{10}H_6O_8S_2Na_2.2H_2O$) and dipyrone ($C_{13}H_{16}N_3NaO_4S$) were obtained from Sigma-Aldrich®. Distilled water was obtained from a glass distillation device.

Pharmaceutical dosage forms of dipyrone were purchased from reliable drugstores. The commercial tablets comprising dipyrone tested were: - Novalgina® (500 mg); Conmell® (320 mg); Anador® (500 mg); Lisador® (500 mg); Buscopan® (250 mg) and Generic Medley® (500 mg).

Solutions

the oxidation probably begins with the isomeric methoxy form of the pyrazolone [22]. The formaldehyde formed is identified by reacting it with warm chromotropic acid, yielding a red-violet color [22]. The nature of this chromogen has never been unambiguously determined but some experimental evidence suggests the hypothesis that it has a mono-cationic dibenzoxanthylium structure [26]. In this work, we use the highly exothermic hydration process of the sulfuric acid and its very low heat capacity to provoke the necessary temperature increase for the reaction to occur [23]. In order to accelerate the reaction iron III was used as catalyst. A drop of a 0.1 mol L^{-1} iron (III) chloride solution was added directly in the solution containing the dipyrone with the chromotropic acid. When the sulfuric acid is added, the increase of the reaction rate can be visually observed when compared to the reaction without the addition of iron III.

The absorption spectrum of the reaction product was obtained using the final solution in the volumetric flask. With the defined working conditions the maximum absorption wavelength was observed at 576 nm.

The stability of the product formed was studied over time. Measurements performed 15 days later showed that the reddish-violet compound formed is completely stable in this time interval when stored at ambient temperature (*ca*. 25 °C).

The molar ratio of the analyte to the analytical reagent and the volume ratio of the concentrated sulfuric acid were investigated. The best conditions were: 1:10 ratio of dipyrone to chromotropic acid using 500 μL of concentrated sulfuric acid.

The absorbance was measured at 576 nm using the volumetric flask as spectrophotometric cell. These results were compared with those obtained with spectrophotometric cuvettes.

The calibration curves were constructed in the range from 1.0×10^{-3} mol L^{-1} to 6.0×10^{3} mol L^{-1} considering the concentration in the aliquot. They can be described by the equations: a) **A** = 0.15 + 110 **C** (r=0.999) where **A** is the absorbance at 576 nm and **C** is the concentration in mol L^{-1} in the aliquot; when using the spectrophotometer with the straight walled volumetric flask and b) **A** = 0.16 + 114 **C** (r=0.998) with the cuvettes.

For both procedures, using the volumetric tubes for spectrophotometric measurements or using the cuvettes, the instrumental limits of detection, LOD, and of quantitation, LOQ, are: LOD $\cong 1.4 \times 10^{-4}$ mol L^{-1} (LOD $\cong$ 3.3 SD / slope); limit of quantitation LOQ $\cong 4.5 \times 10^{-4}$ mol L^{-1} (LOQ $\cong$ 10 SD / slope). The visual qualitative limit of detection is about 5×10^{-6} g of dipyrone in the aliquot.

Alternatively, in order to obtain results more rapidly, a simple proportional calculation can be done using as standard a solution prepared with the expected concentration of dipyrone in the sample. If the obtained result for the sample is within the established limits, the pharmaceutical preparation can be considered in conformity with the pharmacopoeia recommendations.

In order to test the proposed method six commercial pharmaceutical preparations, purchased in local pharmacies, were analyzed. The results were compared with those obtained with the titrimetric procedure recommended by the Brazilian Pharmacopoeia [1], using the paired Student's statistical ***t*** test and the **F** test [24]. As it can be observed in Table 1, for a confidence coefficient of 0.05, in four cases values of ***t***, slightly higher than the theoretical value, were obtained. However, the **F** test shows complete agreement in all cases. For the confidence coefficient of 0.01 complete agreement was achieved in the Student's ***t*** and in the **F** tests.

Resumo: Um método "spot-test" qualitativo e seqüencialmente quantitativo é proposto para análise de dipirona em fármaco "puro" e em preparações farmacêuticas. A formação de coloração vermelho-violeta indica um resultado qualitativo positivo. Na seqüência, um procedimento quantitativo pode ser realizado no mesmo frasco. Os resultados quantitativos obtidos foram comparados estatisticamente com os resultados obtidos pelo método indicado pela Farmacopéia Brasileira, utilizando o teste ***t*** de Student e o teste ***F***. Considerando a concentração em uma alíquota de 100 µL, o limite qualitativo visual de detecção foi de cerca 5×10^{-6} g; instrumentalmente o limite de detecção foi de LOD $\cong$ 1.4×10^{-4} mol L^{-1} e o limite de quantificação de LOQ $\cong$ 4.5×10^{-4} mol L^{-1}.

Palavras-chave: dipirona, spot-test, análise, qualitativa, quantitativa

References

[1] Brazilian Pharmacopoeia, 3rd ed., Organização Andrei Editora, São Paulo, Brazil, 1977, p 406-8.
[2] I.M. Benseñor, São Paulo Med. J., 119 (2001) 190.
[3] H. Ergün, D. A. C. Frattarelli and J. Aranda, J. Pharmaceut. Biomed., 35 (2004) 479.
[4] P. Danielli, and M.B Leal, Rev. Bras. Farm., 84 (2003) 17.
[5] Y. Bentur, and O. Cohen, J. Toxicol.-Clin. Toxic., 42 (2004) 261.
[6] S. C. Pierre, R. Schmidt, C. Brenneis, M. Michaelis, G. Geisslinger, and K. Scholich, Br. J. Pharmacol., 151 (2007) 494.
[7] G. Suarez-Kurtz , F.M. Ribeiro, R.C.E. Estrela, F.L. Vicente, and C J. Struchiner, Braz. J. Med. Biol. Res., 34 (2001) 1475.
[8] Brasil, Anvisa, Agência Nacional de Vigilância Sanitária, Rev. Saúde Pública, 38 (2004) 748.
[9] F.G.D. Vieira, J. M. Crubellate, I.G. Silva, and W.R. Silva, RAE eletron.,1 (2002) 1.
[10] H. Senyuva, I. Aksahin, S. Ozcan, and B.V. Kabasakal, Anal. Chim. Acta, 547 (2005)73-7.
[11] K.A. Sakiara, L. Pezza, C. B. Melios, H. R. Pezza, and M. Moraes, Farmaco, 54 (1999) 629.
[12] J. L. F. C. Lima, S. M. O. Sá, J. L. Santos, and E. A. G. Zagatto, J. Pharmaceut. Biomed., 32 (2003)1011.
[13] L. H. Marcolino Jr, R. A. Souza, O. Fatibello Filho, and F.C. Moraes, Anal. Lett., 38 (2005) 2315.
[14] A.V. Pereira, L. Penckowski, M. Vosgerau, and M. F. Sassá, Quím. Nova 25 (2002)553.
[15] A. P. Nascimento, M. G. Trevisan, E. R. M. Kedor-Hackmann, and R. J. Poppi, Anal. Lett., 40 (2007) 975.
[16] J. S. Albuquerque, V. L. Silva, F. Lima, A. Araújo, and M. C. B. S. M.Montenegro, Anal. Sci., 19 (2003) 692
[17] T. R. L. C. Paixão, C. R. Matos, and M. Bertotti, Talanta, 61 (2003) 725.
[18] E. P. Medeiros, S.L. Castro, F. M. Formiga, S. R. B. Santos, M. C. U. Araújo, and V. B. Nascimento, Microchem. J., 78 (2004) 91.
[19] L. H. Marcolino Jr, V. G. Bonifácio, and O. Fatibello Filho, Quím. Nova, 28 (2005) 783.
[20] M. F. S Teixeira, L. H. Marcolino Jr., O. Fatibello Filho, E. R. Dockal, and E. T. G. Cavalheiro, J. Braz. Chem. Soc., 15 (2004) 803.
[21] P. L.Weinert, L. Pezza, and H.R. Pezza, J. Braz. Chem. Soc., 18 (2007) 846
[22] F. Feigl, Spot Tests in Organic Analysis, 7th ed., Elsevier, Amsterdan, 1966, p. 434-36 and 635.
[23] L. Pezza, M. Tubino, C. B. Melios, and H. R. Pezza, Anal. Sci., 16 (2000) 313.
[24] K. Eckschlager, Errors, Measurement and Results in Chemical Analysis, Van Nostrand Reinhold Company, London, 1972, p. 109-120.
[25] P. Karrer, Organic Chemistry, 4th ed.; Wiley, New York, 1950, p. 798.
[26] P. E. Georghiou, and C.K. Ho, Can. J. Chem., 67 (1989) 871