Development and validation of TLC methods for assessing the authenticity of cardamom raw materials

O.V. Evdokimova, I.A. Tarrab
(GBOU VPO First Moscow State Medical University.
THEM. Sechenov, Moscow)

Development and validation of TLC method for the identification of raw Cardamon

OV Evdokimova, IA Tarrab
Medical University First MGMU them. IM Sechenov (Moscow, Russia)

SUMMARY

National quality standards for medicines should contain a section "Qualitative reactions". This article proposes methods for determining the authenticity of cardamom raw materials for lipophilic and phenolic substances by chromatography in a thin layer of sorbent.

Key words: cardamom, identification of authenticity, chromatography ina thin layer of sorbent.

RESUME

National standards for the quality of medicines should contain a section "Identification". In this article, methods of identification of raw materials cardamom on the lipophilic and phenolic substances by thin layer chromatography of the sorbent are proposed.

Keywords: cardamom, identification, thin-layer chromatography method.

Introduction

In foreign pharmacopoeias, monographs are given on medicinal plant raw materials - Cardamom fruits [4, 5, 8, 10] and Cardamom seeds [11]. In our country, the raw material of cardamom is currently not official [1], however, oil, hydroalcoholic extracts obtained from cardamom are widely studied. This type of raw material can expand the range of domestic medicinal plant materials [2, 3]. The purpose of this work was the development and validation of methods for determining the authenticity by TLC for inclusion in the draft regulatory documentation for raw cardamom.

Materials and research methods

The objects of research were industrial samples of cardamom fruits that meet the requirements of GOST 29052-91 “Spices. Cardamom. Technical conditions ". In medical practice, cardamom seeds are used [1, 4, 5, 8, 10, 11], which are extracted from the fruit immediately before use. We examined samples of fruit, seeds and cardamom fruit capsule to establish the possibility of identifying cardamom seeds. This is important in cases where the powder of cardamom seeds contains an impurity, for example, capsules, and this fact cannot be determined by external signs.

Chromatography in a thin layer of sorbent was performed on TLC Silica gel 60 F254 Aluminum sheets (MERCK, Germany). Extraction of biologically active substances from raw materials was carried out with ethyl alcohol 96%. When developing the method, the optimal separation conditions were selected, including the analysis of the mobile phases used in the analysis of raw materials containing lipophilic and phenolic compounds [6, 7, 9, 11]. As reference solutions: in the analysis of lipophilic substances, solutions of standard samples of eugenol and cineole were used, in the analysis of phenolic compounds - solutions of standard samples of rutin, hyperoside, caffeic acid and chlorogenic acid. We used solutions of anisic aldehyde (for lipophilic substances) and diphenylboryloxyethylamine in 96% ethyl alcohol (for phenolic compounds) as detecting reagents.

Research results and their discussion

The studies carried out made it possible to establish that the best separation of lipophilic compounds of the cardamom raw material was achieved in the methylene chloride - toluene system (1: 1). It is better to separate phenolic substances in the system acetone - ethyl acetate - toluene - water - anhydrous formic acid (20: 10: 10: 5: 5). After the solvent front had passed a distance of 8 cm from the starting line, the plate was removed from the chamber and dried to remove traces of solvents under draft (at room temperature).

When determining lipophilic substances: the plate was treated with a solution of anisaldehyde and dried in a fume hood, and then heated in a drying cabinet at 105–110-C for 2–3 min. and immediately (immediately) viewed in daylight.

On the chromatogram of a solution of standard samples of eugenol and cineole, 2 zones were found: a brownish-violet zone with R_f about 0.45 (eugenol), taken as R_s = 1.0, as well as a blue-violet or purple zone with R_s (for eugenol) about 0.55 (cineole). On the chromatogram of cardamom fruit and seed extraction, 9 zones of brownish-violet, blue-violet or violet color with R_s (for eugenol) about 0.25; 0.45; 0.6; 0.75; 1.0; 1.2; 1.7; 1.9 and 2.1. There may be a brown stripe on the start line and other areas.

On the chromatogram of extraction from cardamom capsules, 9 zones were found: one zone of green or yellow-green color with R_s (eugenol) about 0.2 and 8 zones of brownish-violet, blue-violet or violet color with R_s about 0.25; 0.45; 0.6; 0.75; 1.0; 1.2; 1.7 and 1.9. There may be a brown stripe on the start line and other areas.

It is not possible to distinguish between fruits and seeds of cardamom by the presence of the main zones of lipophilic substances. In the event that, for example, the powder of cardamom boxes is presented as a powder of seeds or fruits of cardamom, this fact can be established by the presence of a zone of green or yellow-green color with R_s (eugenol) about 0.2 and no brownish-violet, blue-violet or violet zones with R_s about 2.1. When determining phenolic substances: the plate was treated with a 1% solution of diphenylboryloxyethylamine in 96% alcohol, dried in a fume hood, and then treated with a 5% PEG solution in 96% alcohol

and immediately dried in a drying oven at 100–105 ° C for 3–5 min, viewed in UV light at a wavelength of 365 nm.

On the chromatogram of a solution of standard samples of caffeic acid, hyperoside, chlorogenic acid and rutin, 4 zones were found: two zones of yellow, yellow-brown or yellow-green color with R_f about 0.45 (hyperoside) taken as R_s = 1.0, and with R_s (by hyperoside) about 0.7 (rutin), two blue zones with R_s about 0.85 (chlorogenic acid) and with Rs about 1.65 (caffeic acid). The chromatogram of cardamom fruit and capsule extraction revealed 4 zones: one zone of yellow, yellow-brown or yellow-green color with R_s (by hyperoside) about 0.7, two blue zones with R_f about 0.85 and 1.65, one yellow-brown zone with R_f about 1.75. Other zones are possible.

The chromatogram of cardamom seed extraction shows one zone with R_f (by hyperoside) about 1.65.

By the presence of only one zone on the chromatogram of cardamom seeds, it is possible to distinguish seeds from bolls and cardamom fruits. The developed methods were validated according to the specificity and suitability of the chromatographic system. The specificity of the methods was assessed by the coincidence of the chromatographic profiles of various batches of raw materials, by the main zones with each other and their correspondence to the description of the method. The number of tested batches of raw materials was at least 3. Chromatographic profiles of different batches coincided in the main zones with each other and corresponded to the description of the methods. The resolution between the zones of standard samples of eugenol and cineole with R_s about 1.0 and R_s about 0.55, respectively.

The resolution between the indicated zones was calculated by the formula: $R = 2 (t_{R2} - t_{R1}) / W_{b1} + W_{b2}$where t_{R1} - distance from the start line to the middle of the cineole zone with R_s about 0.55mm; t_{R2} - distance from the start line to the middle of the eugenol zone with R_s about 1.0 mm; W_{b1}, W_{b2} - the distance between the upper and lower boundaries of each of the indicated zones (width of the zones), mm.

The resolution value between the indicated zones was at least 1.5 (table. 1).

Table 1

Suitability of the chromatographic system

№ п/п	Сотрудник, проводивший валидацию	Значение				
		t_{R1}	t_{R2}	W_{b1}	W_{b2}	R
1	Химик-аналитик 1	21	39	4	5	4
2	Химик-аналитик 2	20	37	4	6	3,4

As an indicator of the suitability of the chromatographic system in the case of identification of phenolic compounds, we chose the resolution between the zones of standard samples of hyperoside and rutin with Rs about 1.0 and Rs about 0.7, respectively.

The resolution between the indicated zones was calculated according to the above formula, where tR1 is the distance from the start line to the middle of the routine zone with Rs about 0.7 mm; tR2 is the distance from the start line to the middle of the hyperoside zone with Rs about

1.0 mm; Wb1, Wb2 - distance between the upper and lower boundaries of each of the indicated zones (zone width), mm. The resolution value between the indicated zones was not less than 1.5 (Table 2).

conclusions

1. Developed and validated methods for the determination of lipophilic and phenolic compounds in raw cardamom by TLC.

2. The studies carried out made it possible to establish chromatographic characteristics of cardamom raw materials and identify cardamom seeds.

3. The results obtained can be included in the FS project for promising type of medicinal herbal raw materials.

table 2

Suitability of the chromatographic system

№ п/п	Сотрудник, проводивший валидацию	Значение				
		t_{R1}	t_{R2}	W_{b1}	W_{b2}	R
1.	Химик-аналитик 1	24	35	2	4	3,3
2.	Химик-аналитик 2	23	34	2	4	3,7

Literature

1. Russian Pharmacopoeia (published by the Medical Council of the Ministry Internal Affairs). 6th ed. - SPb: Edition of K.L. Rikker, –1910. - 592 p.

2. Smirnova Yu.A. Development of scientific and methodological approaches to expansion nomenclature of domestic officinal medicinal plant raw materials: abstract of dis. ... Candidate of Pharmaceutical Sciences. - M., 2009 - 24 p.

3. Tkachenko K.G. Essential oil plants and essential oils: achievements and prospects, modern trends in study and application // Bulletin of the Udmurt University. - 2011. - issue 1. - p.88-100.

4. British Pharmacopoeia 2009. British Pharmacopoeia Volume III. Herbal drugs and Herbal Drug Preparations. Cardamom Fruit.

5. Deutsche Arzneibuch 2008. Amtliche Ausgabe. - Stuttgart: Deutscher Apotheker Verlag, 2008 .-- 410 s.

6. Eke Reich, Anne Chili High-performance thin-layer chromatography for the analysis of medicinal plants // Thyme Medical Publishers, Inc. NY10001. - 2006 .-- 264 s.

7. European Pharmacopoeia, 6th edition, 2008.

8. Pharmacopoeia of the People's Republic of China. Vol. I (English ed.). Beijing, Chemical Industry Press, 1997.

9. Teedrogen und Phytopharmaka: ein Handbuch fur die Praxis auf wissenschaftlicher Grundlage / hrsg. von Max Wichtl. Unter Mitarb. Von Franz-Christian Czygan ... 3, erw. und vollst. uberarb. Aufl. - Stuttgart: Wiss. Verl.-Ges., 1997. 668 s.

10. The Japanese pharmacopoeia: Official from march 31, 2006; English version. - 15th ed. - Tokyo: The Ministry of Health, Labor and Welfare, 2006 .-- 1788 p.

11. United States Pharmacopeia 30 - National Formulary 25. The Official Compendia of Standards. - Official May 1, 2007. - CD-ROM version.

Author's address

K.Pharm.n. Evdokimova O.V., Associate Professor of the Department of Pharmacy oevdokimova@umail.ru

Evdokimova, O. V. Development and validation of TLC methods for assessing the authenticity of cardamom raw materials / O.V. Evdokimova, I.A. Tarrab // Traditional Medicine. - 2013. - No. 4 (35). - S.38-40.