



Trade Science Inc.

ISSN : 0974-7419

Volume 12 Issue 5

Analytical CHEMISTRY

An Indian Journal

Note

ACAIJ, 12(5) 2013 [194-197]

Preparation of high purity analytical standards using high performance liquid chromatography in analytical scale

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ABSTRACT

Obtaining standards with high purity and reliability is certainly the greatest difficulty and source of errors in analytical chemistry. This study aimed to demonstrate the feasibility of obtaining substances with high purity and in sufficient quantities for use as standards in analytical high performance liquid chromatography (HPLC), in a quickly and practical way. Standards were isolated from three classes of substances of interest in the food industry (isoflavones, anthocyanins and carotenoids) from natural sources, in sufficient quantities to build external calibration curves within the range of work required. © 2012 Trade Science Inc. - INDIA

KEYWORDS

HPLC;
Carotenoids;
Isoflavones;
Anthocyanines;
Standards preparations.

INTRODUCTION

The major difficulty in the quantitative analysis by HPLC is to obtain reliable analytical standards. Standardization is certainly the largest source of analytical errors, because it directly impacts the final result^[4]. The acquisition of standards with high purity and certification almost always depends on imports and has high costs. In some cases, the substances are not even available commercially.

In general, standards are sold in small quantities, which makes difficult the qualitative analysis and verification of its purity, essential steps to guarantee the analytical results.

The storage conditions are also critical for main-

taining the chemical stability of standards, especially when dealing with labile substances. Thus, the import process, typically time-consuming, is also a source of uncertainty in the quality assurance of the substance acquired even if dealing with reputed company with certificate of analysis, because there is still the risk of the substance being compromised due to inadequate conditions of shipping and handling.

It is possible to obtain calibration curves with optimal linear coefficients, accurate and reproducible results, but with compromised accuracy in the case of using the substance degraded in standardization. Furthermore, the degradation products of the standards cannot be detected by the method used.

The preparation of the standards in the laboratory

of analysis is an alternative. Chromatography in preparative scale, in open column^[4] or preparative chromatography systems can be used for this purpose. Large amounts of standards with high purity can be obtained, however, the technique requires expensive equipments (preparative systems), demands large amounts of solvents, and especially longer time procedures.

For the construction of external calibration curves in HPLC, the standard prepared or acquired is weighed and then diluted to various concentrations that perform the range of the method. However, the actual quantities injected into the chromatographic systems are generally in the order of ng or even pg (10^{-6} g).

The objective of this preliminary work is to demonstrate the possibility of obtaining analytical standards in sufficient quantity and with high purity using the analytical scale by HPLC technique with non-destructive detection. Is still feasible to obtain these standards from the own matrices analyzed.

EXPERIMENTAL

The methodology of standards isolation by HPLC was applied to the preparation of analytical standards for three classes of substances of interest in the food industry: isoflavones, carotenoids and anthocyanins. All prepared standards were obtained using an analytical chromatograph Waters Alliance™ 2695 with photodiode array detector Waters 2996 and a six-way valve from Rheodyne™, coupled to the output of the detector. Substances of interest were collected at the output of the detector when eluting, which was done manually or with the use of the Rheodyne™ valve controlled by the Empower™ software.

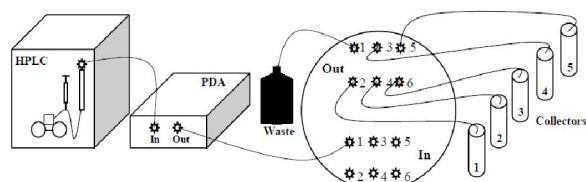


Figure 1 : Scheme of the chromatographic system with Rheodyne™ valve.

The Rheodyne™ valve was configured such that the position 1 leads the detector effluent to waste, while positions 2 through 6 lead it for five collectors (Figure

1). With this configuration is possible to collect up to five substances in each chromatographic injection, automatically.

Standards of isoflavones were obtained from methanol extracts of crushed soy germ (hypocotyl)^[2]. The extract was concentrated and isoflavones separated by C_{18} HPLC column with gradient elution of methanol in water. Rheodyne valve was used for the automatic collection of three peaks corresponding to the glycosidic isoflavones daidzin, genistin and glycitin.

In the preparing of anthocyanins standards^[3], methanol extracts of açai (*Euterpe oleracea* Mart.) were used. Two peaks related to the anthocyanins cyanidin-3-glucoside and cyanidin-3-rutinoside were isolated.

To obtain standards of carotenoids were used as matrices carrot and pumpkins^[1], for the preparation of α -carotene and β -carotene; pulp of cajá (*Spondias mombin* L.) to prepare standards of β -carotene, β -cryptoxanthin and zeinoxanthin^[7,8] and lettuce for the isolation of *trans* isomers of β -carotene, 9-*cis*- β -carotene and 13-*cis*- β -carotene. The prepared extracts^[6] were concentrated and the carotenoids were separated on C_{30} analytical column^[2]. The eluting carotenoids were manually collected in the detector output.

RESULTS AND DISCUSSION

Figure 2 shows the chromatograms and UV/Vis spectra of the three isolated glycosidic isoflavones, daidzin (99.85%), glycitin (99.94%) and genistin (99.95%).

Figure 3 shows the chromatograms and UV/Vis spectra of the two anthocyanins isolated from açai, cyanidin-3-glucoside (96.0%) and cyanidin-3-rutinoside (97.5%).

With five injections of 15 μ L of concentrated extract of carrot were obtained 16.5mg of α -carotene and 19.6mg of β -carotene with purity of 99.2 and 99.1% respectively (Figure 3 A and B). From lettuce were obtained isomers 9-*cis*- β -carotene (98.3%), 13-*cis*- β -carotene (93.0%) and *all-trans*- β -carotene (99.8%) (Figure 3 C and D). Were obtained from cajá β -cryptoxanthin (99.8%) and zeinoxanthin (99.0%) (Figure 3 E and F).

Note

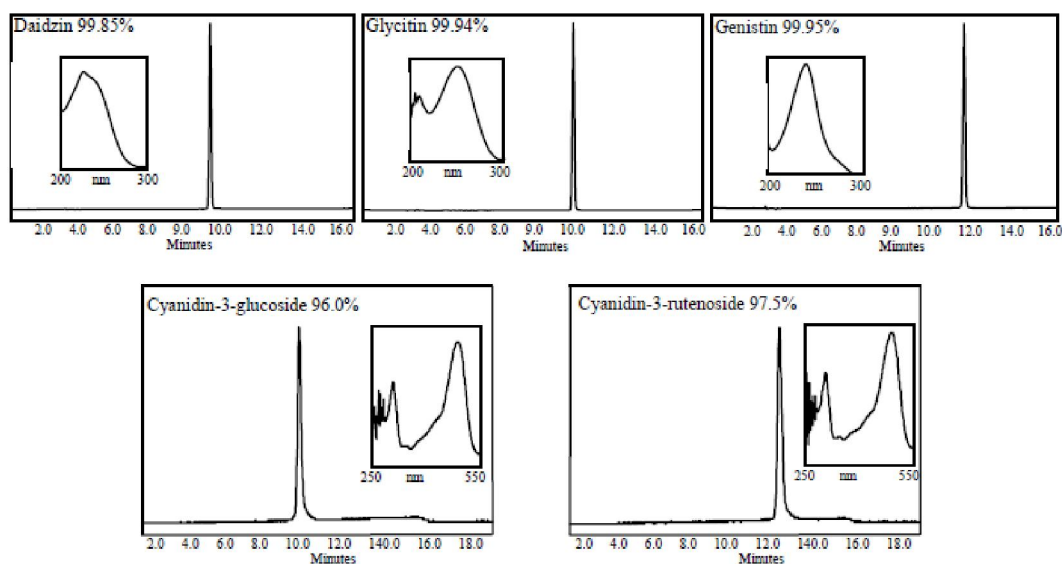


Figure 2 : Chromatograms and UV/Vis spectra of isoflavones and anthocyanins isolated.

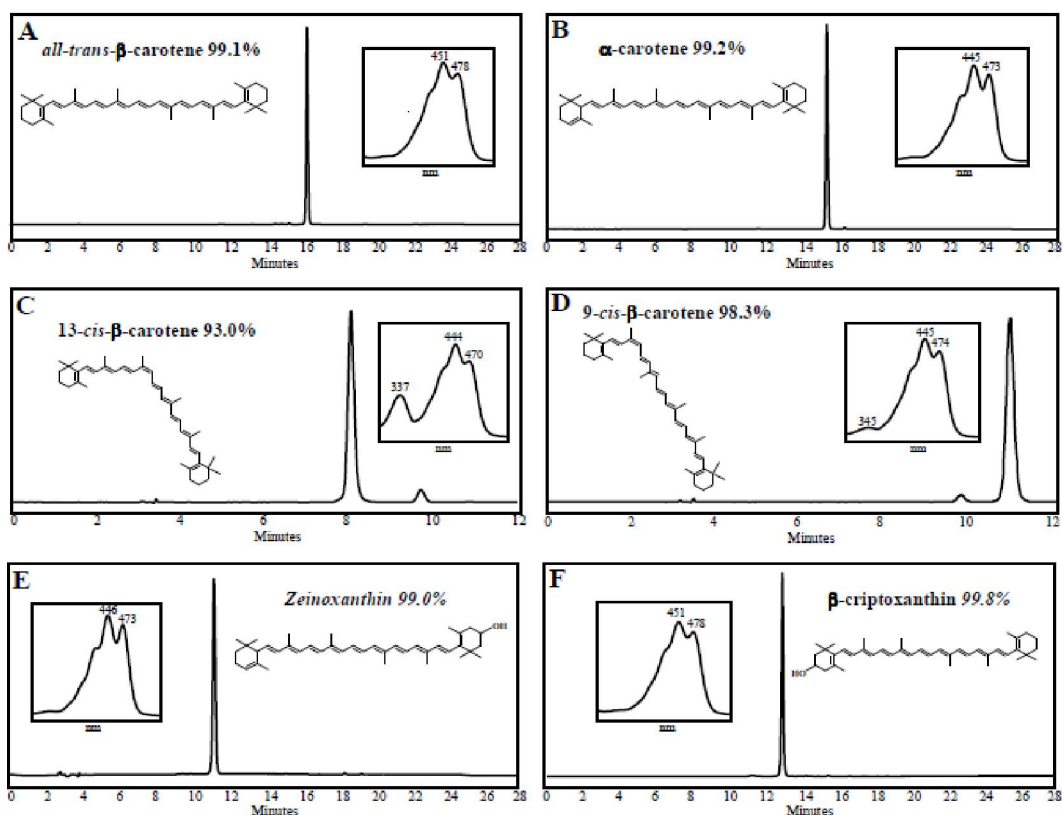


Figure 3 : Chromatograms and UV/Vis spectra of carotenoids isolated.

CONCLUSION

Using HPLC in analytical scale was possible to prepare analytical standards of three isoflavones, two anthocyanins and five carotenoids, including *cis* isomers, in the laboratory, quickly, simply and in sufficient quantities to build external calibration curves.

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