Electronic Supplementary Material (ESI) for Analytical Methods.

Fast simultaneous quantitation of valsartan and amlodipine besylate using Eco-friendly micellar HPLC-UV method: application to spiked human plasma and content uniformity testing for amlodipine

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Supplementary Information

- **Table S1:** Comparison between the reported HPLC methods and the proposed method.
- **Table S2:** Analytical eco-scale for assessment of greenness of the proposed method.
- **Scheme S1:** Greenness of solvents commonly used in analytical chemistry, based on solvent selection guides.
- **Details about Content Uniformity testing.**

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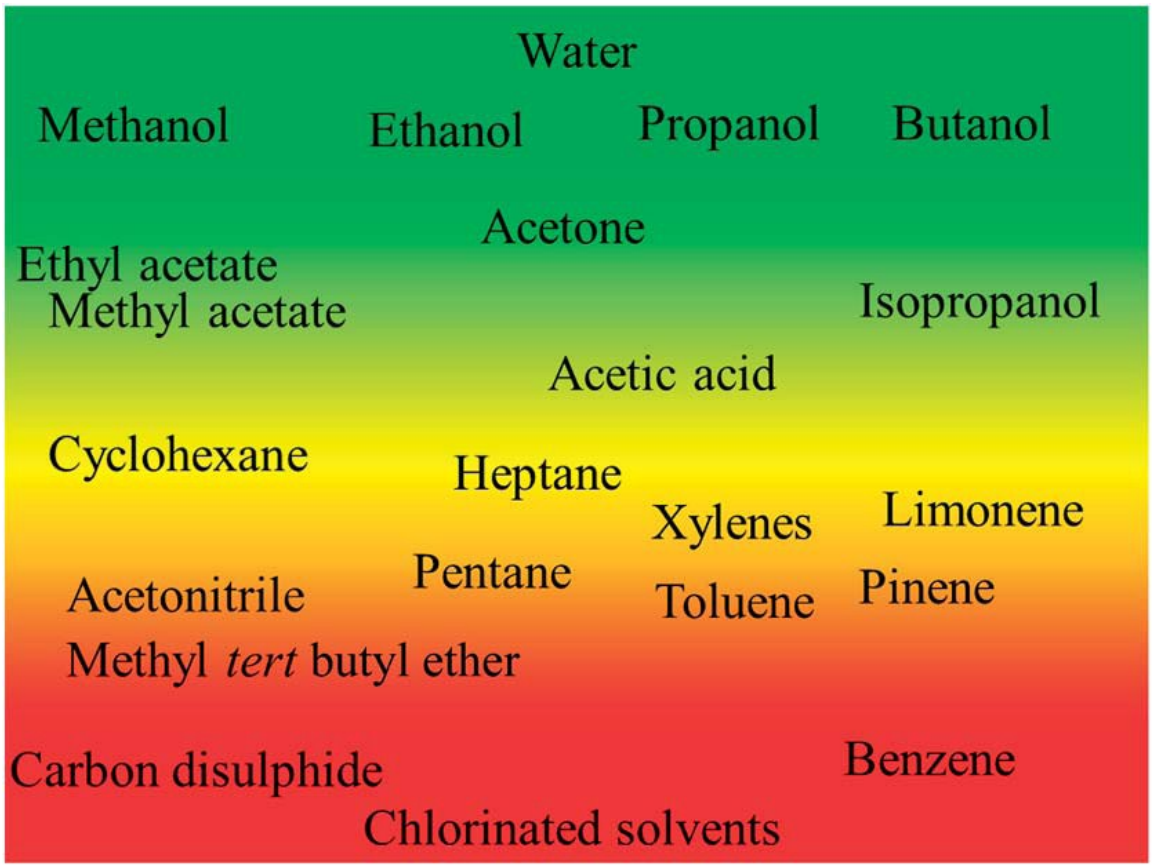
Table S1: Comparison between the reported HPLC methods and the proposed method.

Reference	Range	Mobile Phase	Column	Detection	Run time	Application
4	VAL: 112- 208 µg/mL AML: 07- 13 µg/mL	Methanol: Water (62:38), pH at 3.0	C-18 Column	UV detection at 230 nm	6.0 min	tablets
5	VAL: 160 - 480 µg/ml AML: 10 - 30 µg/ml	dihydrogen phosphate buffer : acetonitrile (55:45), pH 3.2	C-18 Column	UV detection 237 nm	2.0 min.	Tablets and stability study
6	VAL: 0.10 -20 µg/mL AML: 0.01- 0.80 µg/mL	gradient elution with aqueous 0.02% sodium hexane sulfonate–glacial acetic acid (500:1,v/v) : acetonitrile	C-18 Column	on-line Fluorescence detection	15.0 min	Tables and rat plasma
7	VAL: 1–100 ng/mL AML:10–1000 ng/mL	phosphate buffer:acetonitrile:meth-anol (60:30:10), pH 4.0 ± 0.1	Phenyl Column	on-line fluorescence detection	15.0 min	plasma
8	VAL: 32-128 µg/ml AML: 19.6-78.4 µg/ml	water, acetonitrile and glacial acetic acid (300:700:1)	C-18 Column	UV detection at 220 nm	6.0 min	tablets
9	VAL: 5-50 µg/ml AML: 2-20 µg/ml	acetonitrile-phosphate buffer (50/50)	C-18 Column	UV detection at 254 nm.	5.0 min.	Tablets and plasma
Proposed method	VAL: 5-300 µg/ml AML: 5-200 µg/ml	0.16 M sodium dodecyl sulphate (SDS), 0.3% triethylamine (TEA), 15% n-propanol, pH 2.5	Monolithic 18 Column	UV detection at 240 nm.	4.3 min	Tablets, plasma and content uniformity testing for AML

Table S2: Analytical eco-scale for assessment of greenness of the proposed method

Items of the method	Value	Penalty points
Reagent		
n-propanol	10–100 mL	4
TEA	1–10 mL	2
SDS		
o-phosphoric acid	1–10 mL	2
Total penalty points (multiply the reagent amount and reagent hazard)		12
Instruments		
Energy	<1.5 KWH per sample	1
Occupational hazard	Emission of vapours to air	1
Waste	1–10 mL	3
Heater	No heating	0
Conditioning and washing time	Normal	0
Total penalty points (sum of instrument points)		5
Total penalty points		17
Analytical Eco- Scale total score	100 -17	83



Scheme S1: Greenness of solvents commonly used in analytical chemistry based on solvent selection guides

[M. Tobiszewski, *Analytical Methods*, 2016, **8**, 2993-2999].

Content Uniformity Test

Pharmaceutical products prior to their approval for market authorization are evaluated and tested according to their quality test specifications. Tests, described in the quality test specifications contain physical tests (appearance, average mass, etc.), chemical tests (assay, purity, etc.), and pharmaceutical tests (dissolution, content uniformity) [B. Banfai; K. Ganzler; S. Kemeny; *Content uniformity and assay requirements in current regulations.* J. Chromatogr. A 1156 (2007) 206-212]. Assay and content uniformity tests are two major aspects of drug quality assessment. Most quality attributes are regulated in the pharmacopoeias. The content uniformity tests are used for proving the uniform distribution of the active content in a production batch. It is performed by measuring the active content of individual dosage units. The British Pharmacopeia as well as the United States Pharmacopeia uses the same procedure for content uniformity testing.

The acceptance value is calculated from the following formula:

$$\mathbf{AV = |M\text{-}X| + KS}$$

Where:

AV is the acceptance value, **M** is a reference value that equals either;

98.5% if $\bar{X}$ < 98.5% **or,** $\bar{X}$; if $\bar{X}$ is between 98.5–101.5% **or,** 101.5% if $\bar{X}$ > 101.5% where $\bar{X}$ is the mean of individual contents.

k is the acceptability constant (tolerance factor); it equals either; **2.4** if n=10 or **2.0** if n=30.

S is the sample standard deviation.

L1 is the maximum allowed acceptance value; it equals **15.0** unless otherwise specified in the individual monograph of the drug.

L2 is the maximum allowed range for deviation of each dosage unit tested from the calculated value of **M**; it equals 25.0 unless otherwise specified in the individual monograph.

Thus, the acceptance value (**AV**) is calculated as the sum of two components, namely the difference of the observed mean and the reference value $|M - \bar{X}|$and the width of the tolerance interval (**KS**). Thus, the decision on accepting/rejecting a batch depends not only on the width of this interval but also on the shift of the mean from the nominal value.

Due to the high sensitivity of the proposed method and its ability to rapidly analyse a single tablet extract with sufficient accuracy, the method is ideally suited for content uniformity testing. The steps of the test were adopted according to the USP procedure. The acceptance value (AV) was calculated and it was found to be smaller than the maximum allowed acceptance value (L1). The results demonstrated excellent drug uniformity for AML.