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A Simple RP-HPLC Method Development and Validation for the Simultaneous Determination of Vitexin and Isovitexin in Mung bean (*Vigna radiata* L.)

AcSIR

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Contents

Mung bean (*Vigna radiata*)

Belong to the family "Leguminosae".

They are mainly grown for their edible seeds

India is the major producer of mung bean.

Nutritional Importance of Mung bean

S.No.	Component	Quantity
1.	Calorific value (cal./100g)	334
2.	Crude protein (%)	24.0
3.	Fat (%)	1.3
4.	Carbohydrates (%)	56.6
5.	Ca (mg/100g)	140
6.	Fe (mg/100g)	8.4
7.	P (mg/100g)	280
8.	Vitamin B_1 Thiamin(mg/100g)	0.47
9.	Vitamin B_2 Riboflavin(mg/100g)	0.39
10.	Vitamin B_3 Niacin(mg/100g)	2.0

Duh et al 1997, Mubarak 2005, Blessing and Gregory 2010

Pulse Crops, by B.Baldev, S.Ramanujam and H.K.Jain, PP. 563

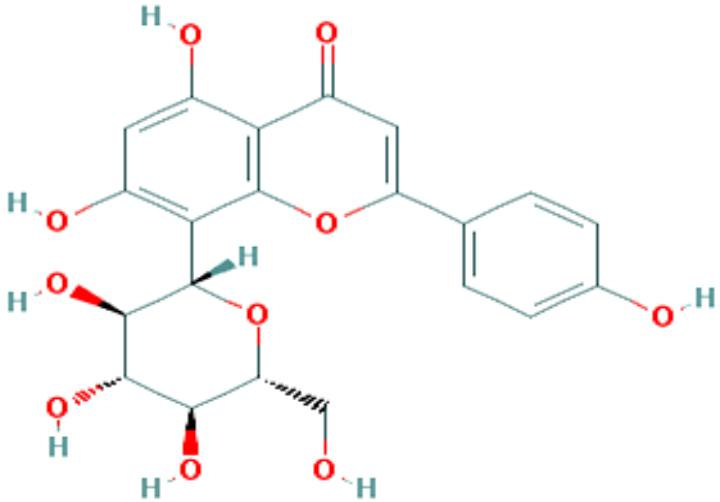
Phenolic Compounds Present in Mung Bean

S.No.	Phenolic compounds	Reference
1	Vitexin	Yao et al 2011, Li et al 2012, Zhang et al 2013
2	Isovitexin	Yao et al 2011, Li et al 2012, Zhang et al 2013
3	*p*-Coumaric acid	Sosulski and Dabrowski 1984, Kim et al 2013, Silva et al 2013, Pajak et al 2014
4	*t*-Ferulic acid	Kim et al 2013, Silva et al 2013, Pajak et al 2014
5	Chlorogenic acid	Kim et al 2013, Silva et al 2013
6	Neochlorogenic acid	Silva et al 2013
7	Catechin	Kim et al 2013, Nair et al 2015
8	Myricetin	Kim et al 2013
9	Quercetin	Lin et al 1997, Kim et al 2013, Pajak et al 2014, Nair et al 2015
10	Kaempferol	Lin et al 1997, Kim et al 2013, Silva et al 2013, Pajak et al 2014
11	Gallic acid	Kim et al 2013, Pajak et al 2014, Nair et al 2015
12	Caffeic acid	Sosulski and Dabrowski 1984, Kim et al 2013, Silva et al 2013, Pajak et al 2014

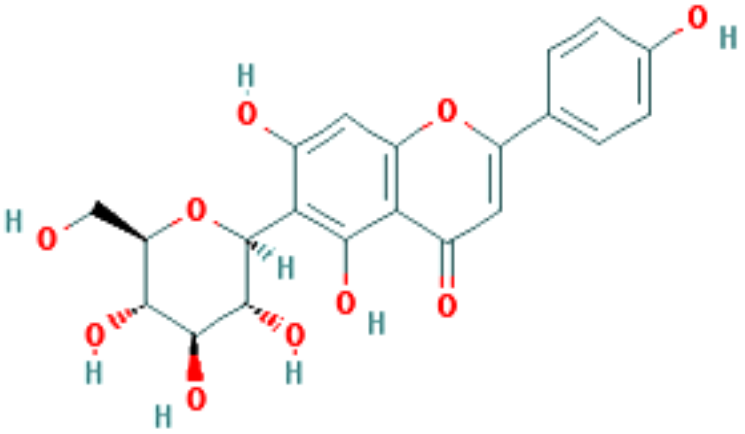
Vitexin and Isovitexin

Flavone C-glucosides

Important constituent of the flavonoid family.



Chemical structures of Vitexin



Chemical structures of Isovitexin

Chemical formula: $C_{21}H_{20}O_{10}$
Molar mass: 432.38 g/mol

Bramati et al 2003, Hien et al 2002

Biological and Pharmacological Importance

- Anti-cancerous
- Hypotensive
- Anti-inflamatory
- Antispasmodic
- Antimicrobial
- Antioxidant/free radical scavenging
- Radioprotective

Prabhakar et al 1981, Agnese et al 2001, Picerno et al 2003, Bramati et al 2003, Hien et al 2002

Literature Review Related to the Quantification of Vitexin and Isovitexin in Different Samples

S.No.	Retention time (min)		Sample	Reference
	Vitexin	Isovitexin		
1	13.2	14.7	Unfermented Rooibos tea	Bramati et al 2013
2	15.8	16.4	Bamboo leaves fortified foods	Zhang et al 2005
3	25.4	27.2	Millet grains	Chandrasekara and Shahidi 2011
4	17.39	18.69	Mung bean	Yao et al 2011
5	20.2	22.9	Mung bean	Li et al 2012
6	28.61	27.16	*Clinacanthus nutans* leaves	Chelyn et al 2014
7	19.8	23.7	*Microctis folium*	Chen el al 2013
8	ND	47.1	*Euterpe oleracea, E. precatoria*	Lisbeth et al 2009
9	39.0	41.0	Unfermented and Fermented Rooibos tea	Stalmach et al 2009
10	8.135	10.868	*Passiflora incarnata*	Grice et al 2006

HPLC system

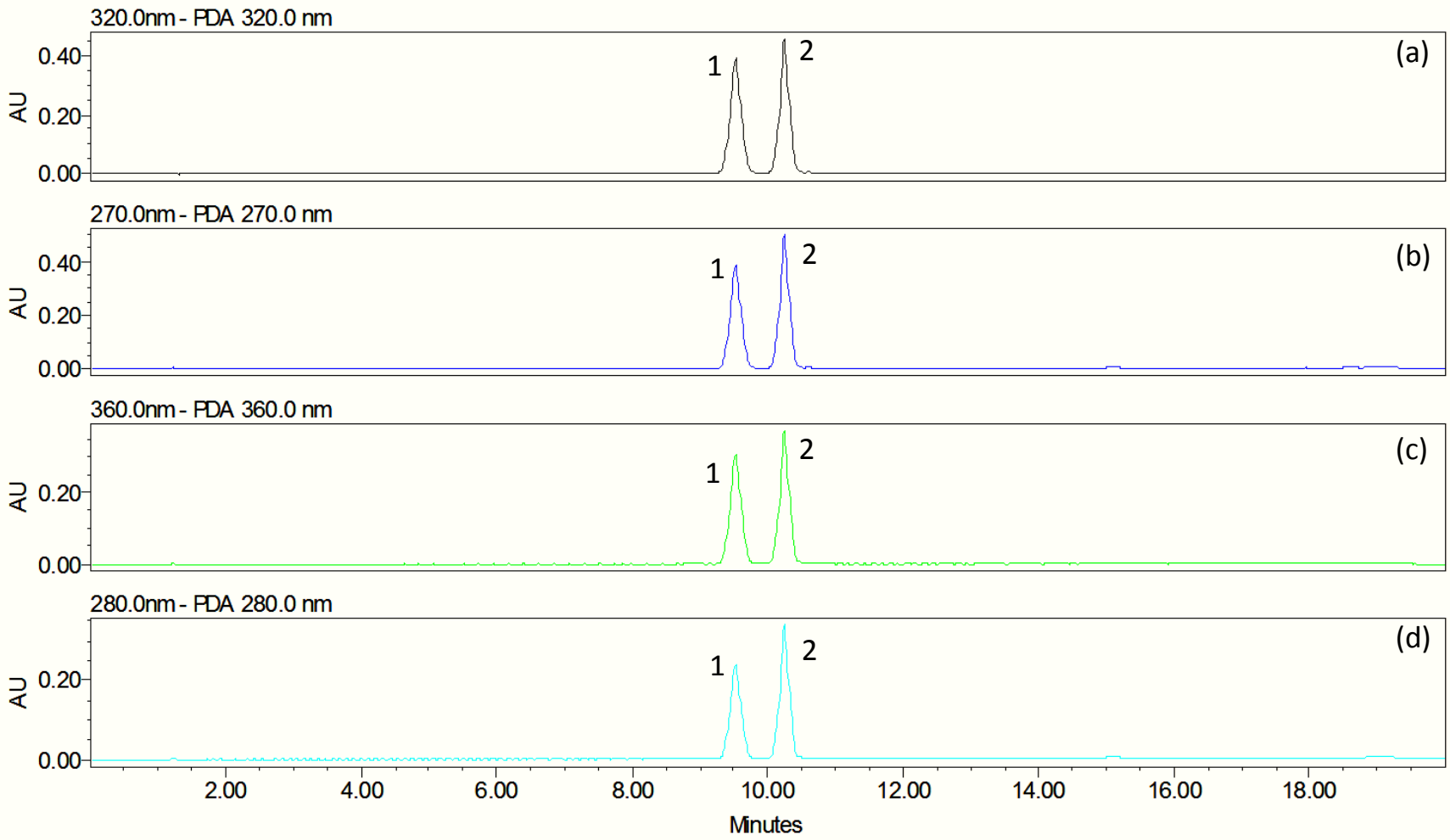
System	Waters HPLC system
Detector	Photo Diode Array
Software	Waters Empower 2
Column	Zorbax SB RP C-18 (250 mm × 4.6 mm × 5 µm pore size)

Chromatographic Conditions and Gradient Used for Proposed Method

Solvent A	0.1% OPA (v/v)
Solvent B	Acetonitrile
Flow rate	1ml/min
Sample volume	20 µl
Column temperature	30˚C
Detector scan range	200–400 nm
Chromatograms extracted	360 nm

Time (mins)	%A	%B
0	95	5
18	64	36
20	95	5

Selection of Detection Wavelength

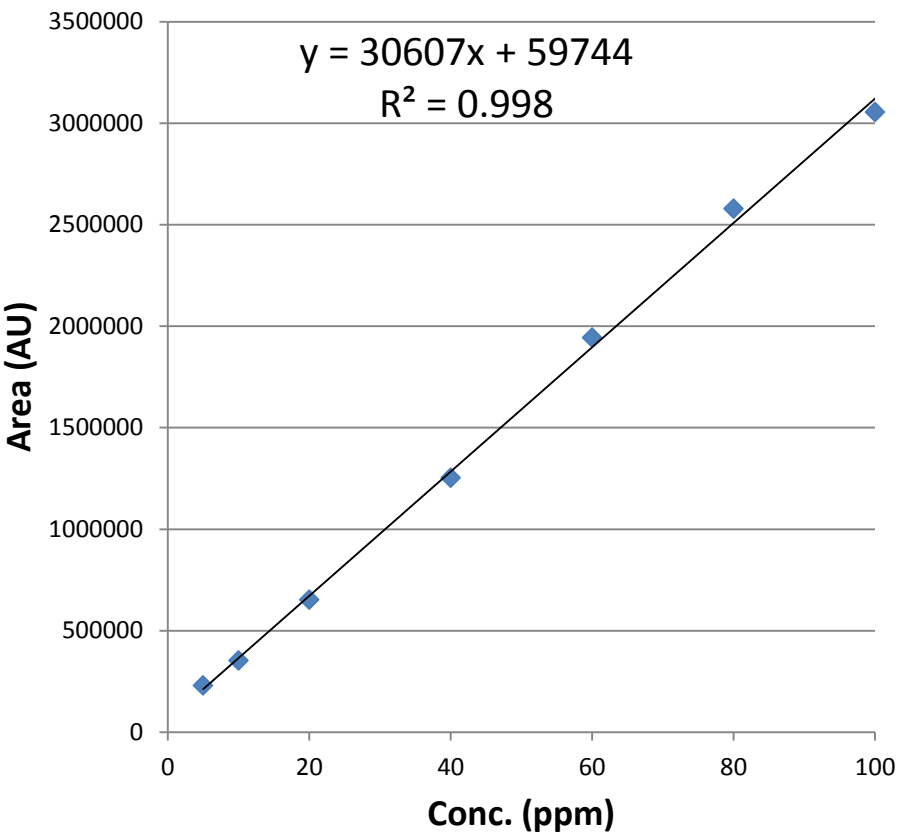


Standard chromatogram of (1) Vitexin and (2) Isovitexin (100 ppm) at (a) 320nm (b) 270nm(c) 360nm (d) 280nm

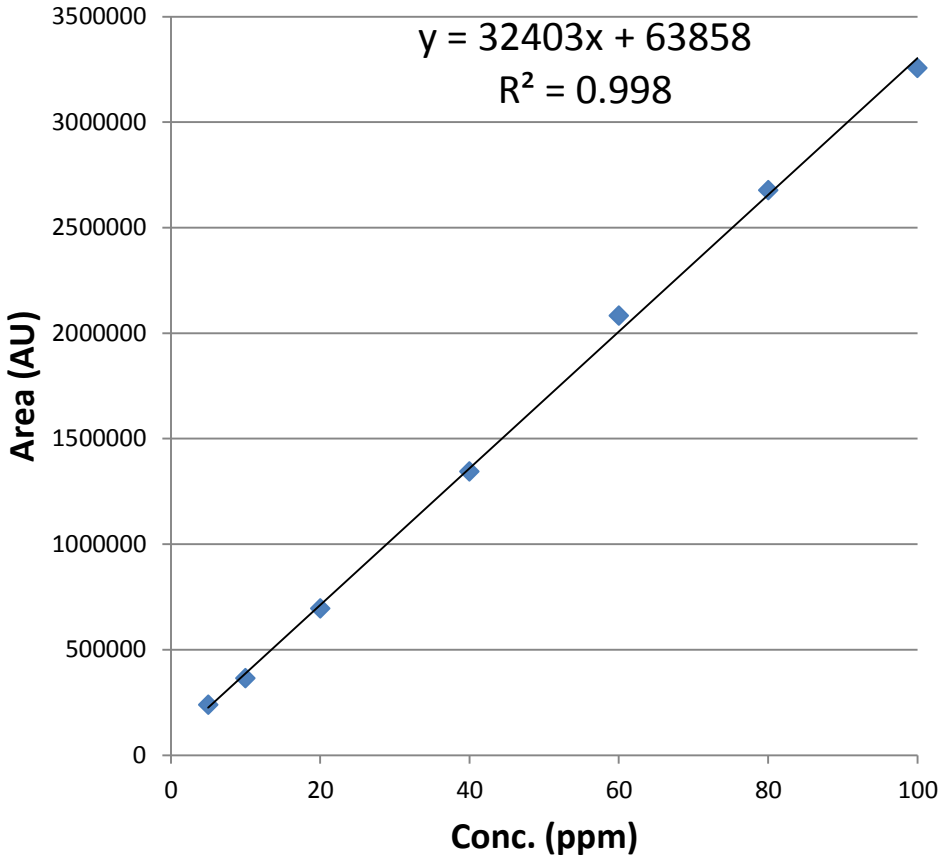
Comparison of Correlation Coefficient at Different Wavelengths

Compound	270nm	280nm	320nm	360nm
Vitexin	0.998	0.997	0.998	0.999
Isovitexin	0.998	0.998	0.998	0.999
Mean±SD	0.998±0	0.9975±0.0007	0.998±0	**0.999±0**

Calibration Curve for the Estimation of Compounds in Samples



Calibration curve for Vitexin



Calibration curve for Isovitexin

UV Spectrum of Vitexin and Isovitexin

UV spectrum of Vitexin

UV spectrum of Isovitexin

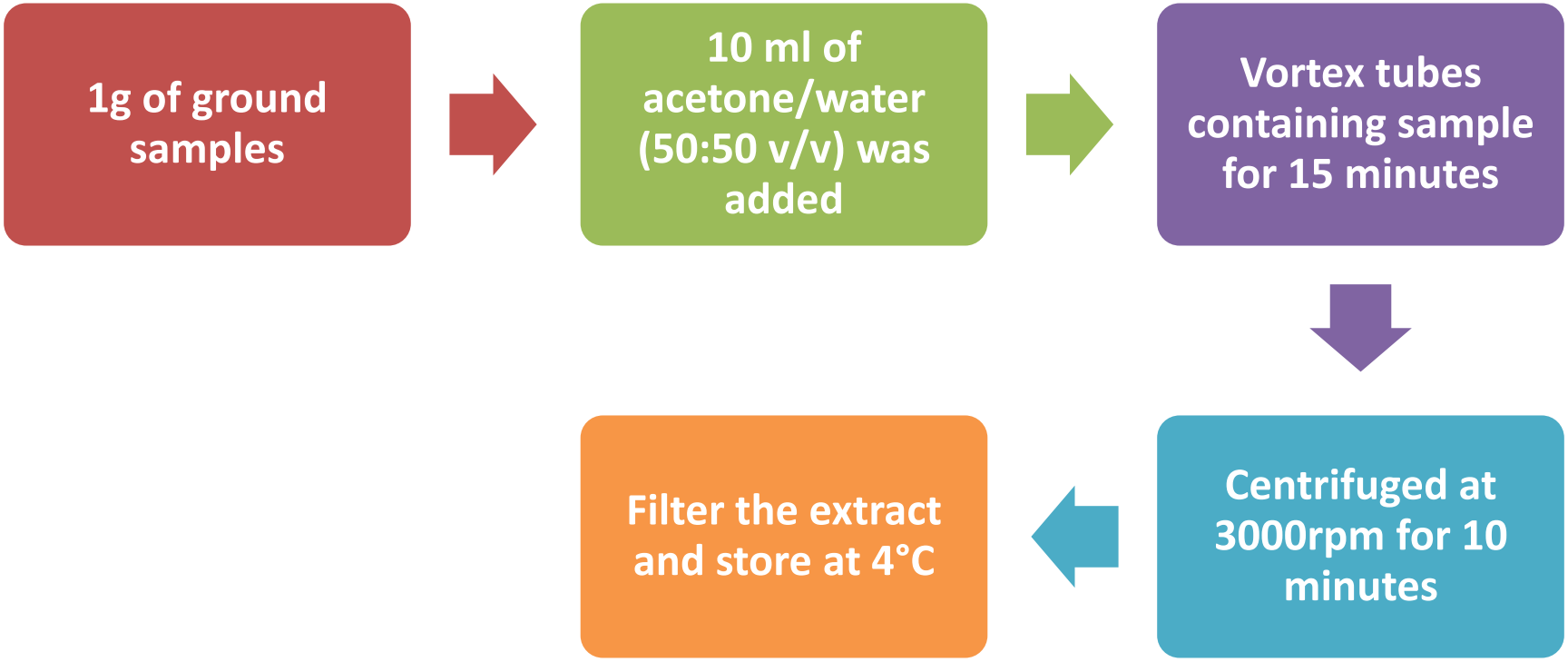
Validation Parameters of Proposed Method

Parameters		Vitexin	Isovitexin
RT (mins)		9.53±0.027	10.23±0.021
Intraday precision		4785865±41181.1 (1.42)	5897217±95696.41 (1.73)
Interday precision		4722883.3± 13013.91 (0.98)	5841429.3±30658.2 (1.20)
Linearity	a	30607	32403
	b	59744	63858
Recovery (%)		98.27±0.63 (0.63)	97.60±0.36 (0.38)
LOD (ppm)		0.94±0.02	0.69±0.02
LOQ (ppm)		3.12±0.07	2.30±0.08

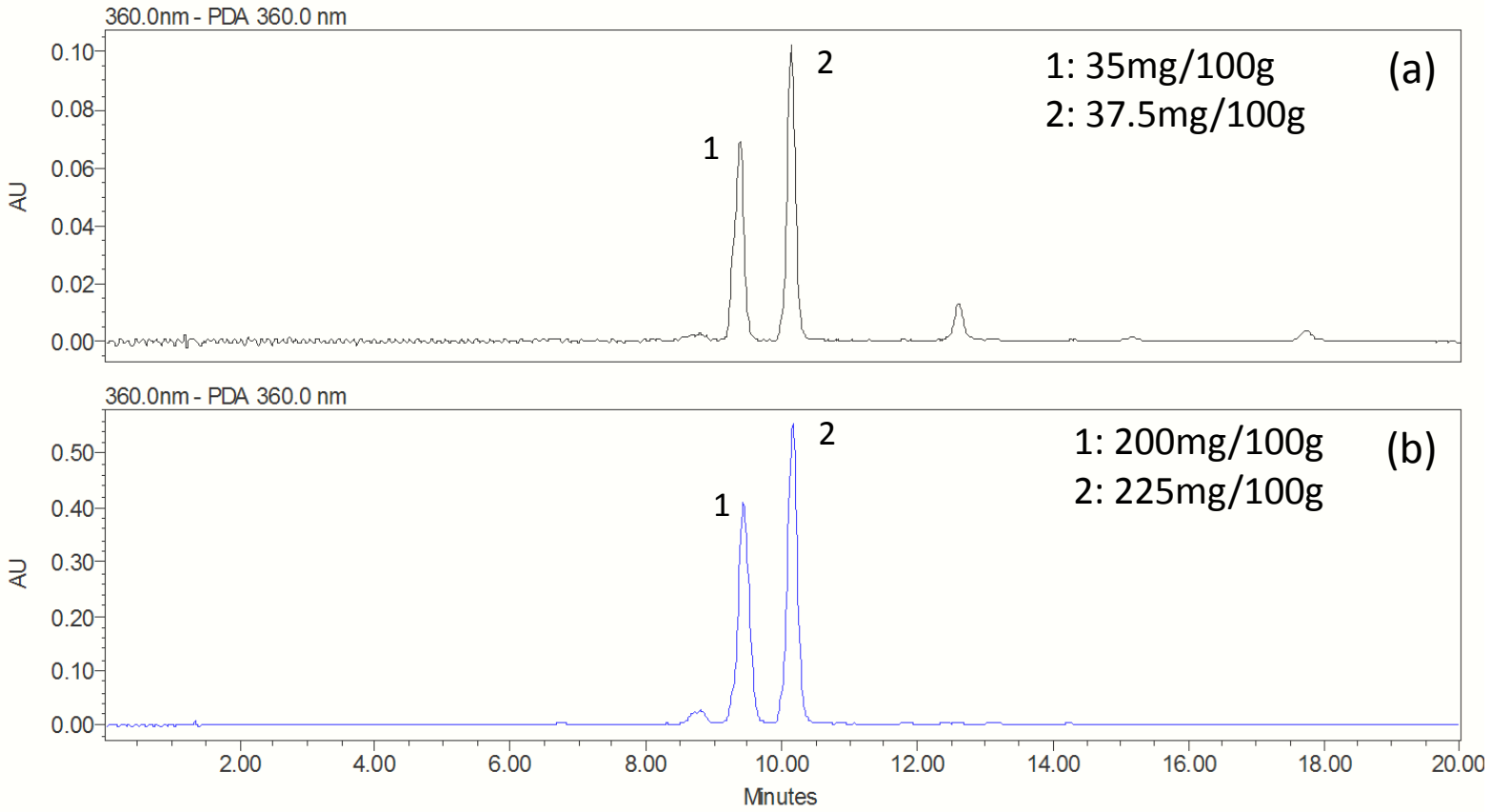
Values are expressed as mean±SD, values in parentheses are % RSD

Extraction of Flavones from Mung bean

Extraction method: Xu & Chang, 2008



Quantification of Mung bean Samples



Chromatogram of (a) sample procured from Ludhiana (b) sample procured from Chandigarh:
1. Vitexin 2. Isovitexin

Conclusions

- The proposed HPLC method is a precise, rapid, sensitive and accurate for the analysis of vitexin and isovitexin in mung bean.
- Effect of different processing technologies and post harvesting techniques can be detected using this method.

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Thank you