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Determination of Sulfur applied on grapes by High-Performance Liquid Chromatography with Diode-Array Detection

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ABSTRACT: A validated high-performance liquid chromatography–diode array detector method for simultaneous determination of the Sulfur on grape was developed. Estimated limit of detection and limit of quantification for Sulfur were 0.01 and 0.05 mg kg^{-1}, respectively. The recoveries were in the range of 96.3%–99.4%. The average initial deposit of in grape fruits and leaves was observed to be 22.4 and 53.70 mg kg^{-1}, respectively at single application rate. The dissipation experiments showed the half-lives ($T_{1/2}$) of Sulfur were around 2.57 and 4.6 days, in grape fruits and leaves samples respectively. According to the maximum residue limit (MRL) the pre-harvest interval (PHI) of Sulfur on grape fruits and leaves was 0 and 2 days after the treatment.

KEYWORDS: Sulfur, grapes , Residues, half-life times, dissipation, HPLC- diode-array.

1.INTRODUCTION:

Historically, studies on sulfur metabolism in plants have been conducted in relation to industrial pollution **(Considine, 2015)**. Many soils now have sulfur deficiencies as a result of the sulfur pollution issue being resolved. There are still important unanswered problems about how various plant organs handle sulfur compounds that are reactive and potentially harmful. We address sulfur dioxide/sulfite absorption in grape berries in this review in light of gene expression and quality characteristics, which are still important to the food business. By contrasting the various reactions in fruit and leaves, we examine the intrinsic metabolism of sulfite and its implications for fruit biology and postharvest physiology. We also draw attention to discrepancies in the definition of "ambient" environmental or industrial SO2 exposures.

Sulfur is one of the most important fungicides and is also widely used as an insecticide. Sulfur is a yellow mineral that is insoluble in water but is soluble to 2.4% in benzene and to 50% in carbon bisulfate **(White-Stevens, 1969)**. Sulfur was the first foliar fungicide. Wet- table sulfur powders or pastes of very fine particle size are widely used to control powdery mildews on fruits and vegetables and as fungicides.

Grape (***Vitis vinifera***) is one of the most widely-grown fruit crop in the world. Thompson Seed less grape cultivar ranking as the most important table grape variety grown in Egypt. Worldwide, the planted areas of grapes are estimated by 24 million feddan and the total yield exceeds than 60 million ton annually. In Egypt, the grape is planted in different type of soils and represents the second position between fruit crops after citrus. In the earliest writings and archives associated to all sorts of agricultural and religious activities, grapes and its products were given a significant place **(Hassan, *et al*, 2013)**. The grape crop is frequently infested by a number of diseases at all stages of its development. The crop is often applied with chemical pesticides to offer protection from severe damage. Limited data have been reported concerning the dissipation of Sulfur fungicide in grape fruit and leaves, as a result, no published data are available concerning the fate of Sulfur in grape. Literature manuscripts report HPLC-UV analytical methods for determination of sulfur in vegetable and fruits after extraction from sample using a mixture of water, methanol, and dichloromethane and clean up by column chromatography with silica gel **(Cassidy, 1976).**

Therefore, the aims of the present study were to evaluate the dissipation of sulfur residues as a function of time and to calculate the PHIs on treated grape fruits and leaves.

2.MATERIALS AND METHODS:

2.1.Chemical and reagents:

All organic solvents were of HPLC grade and supplied by Merck, USA. Methanol dichloromethane, iso-octane, iso-propanol and Silica gel was activated by heating at 200ºC for 4 h in the oven before use and kept in desiccators. A stock standard solution (100 g /ml) was prepared with dichloromethane and stored at -4ºC. The standard working solutions were prepared from stock solution by serial dilution with dichloromethane at 0.01, 0.05, 0.1, 0.2, 0.5, 1.0 and 2.0 g /ml and were stored at 4ºC before use.

2.2.Field experiments:

Grape trees were cultivated in plots, each plot contained 20 vines. Plots were arranged in complete randomized block design at Kaha, Kaliobia Governorate, Egypt, on 2 August 2013. Common agricultural and fertilization practices were used. No fungicides sprays were applied to the test plots prior to or during this experiment. Mature plants were sprayed by commercial formulation of Sulfur (Pandel 8%SC) at the recommended dose (125 g/100 L) using knapsack sprayer motor. The spray solution was prepared in accordance with the manufacture recommendation. The control plots were left unsprayed. There was no rainfall at any time during the experimental period. The average daily temperature during the experiment was from 25 to 39°C.

2.3.Sampling and Storage:

Sampling was performed by randomly collecting from various places of the experimental plots according to the **FAO/WHO (1986)** recommendations. Three replicates were made leaves and fruit samples were taken 0, 1, 3, 5, 7, 12, 15, 21 and 29 days after application. Random samples of about 1 kg were collected from each plot and the samples were transferred immediately to the laboratory in an ice box. The samples were comminuted using the laboratory blender and representative homogenized (100 g) of each sample.

2.4.Analytical Procedure:

Samples were extracted and cleaned up immediately after sampling according to **(NASR, 2010)**.

2.4.1.Extraction: 100 g of the sample material was mixed with 25 ml of methanol and 150 ml of dichloromethane. The mixture was agitated for one hour on agitator. The dichloromethane phase separating from the mixture contains the entire sulfur from the sample and was evaporated to dryness using rotary evaporator. The dry residue was dissolved in 2 ml of (80% iso-octane + 20% iso- propanol).

2.4.2.Clean up:

Small volume of iso-octane was placed in column chromatography (30 cm length and 2.2 cm i.d) containing pieces of glass wool and slurry with 15 g of silica gel activated was made. Then the solvent was drained to the surface of the silica gel layer. The extract solution was transferred into the column. The solution out was washed with 3 ml of the elution mixture (97% isooctane + 3% iso-propanol) and was allowed it to seeped into the tube. Then the sulfur was eluted using the same mixture. The first 25 ml (0-25 ml) was taken and reject it. The main volume of 30 ml (25-55 ml) contains the sulfur. The mixture was taken in a 100 ml round flask and evaporated to dry matter in the rotary evaporator. During analysis the solutions should be exposed to sunlight as little as possible. The residue was redissolved in 2 mL of dichloromethane for analysis by HPLC.

2.5.Instrumental determination:

HPLC analysis was performed with an Agilent 1100 HPLC system (USA), with photodiode array detector. The chromatographic column was C18 Zorbax SB (250 9 4.6 mm, 5 lm film thickness). Flow rate of mobile phase (dichloromethane /Methanol = 5/ 95 v/v) was 1.3 mL/min. and injection volume was 20 uL. Detection wave length for detection of sulfur was set at 260 nm. The retention time of Sulfur was about 8.5 min. Recovery studies were carried out by spiking 3 replicates of untreated date samples (control) with 50, 100, and 50 mg/kg of Sulfur. Samples were analyzed using their prescribed procedure and mean values of the three replicates were calculated.

2.6.Method validation:

The validation of the proposed analytical method (HPLC -DAD) was carried out according to the SANCO document 10684/2009 **(SANCO/10684, 2009)**. Linearity was evaluated by constructing matrix matched calibration curves in the range of 0.1–100 µg / L for HPLC -DAD. Method sensitivity and recovery were determined by using samples spiked with the tested pesticide at three different levels. Fortified samples were extracted as described earlier and the average recovery percentages for fortified samples were determined. Limits of detection (LOD) and quantification (LOQ) were evaluated as the pesticide concentration that produces a peak signal-to-noise ratio of 3/1 and 10/1, respectively.

The rate of degradation (K) and half-life (t1/2) values were obtained from the following equation of **(Gomaa and Belal, 1975)**.

Rate of degradation (K) =2.303 X slope

Half-life (t1/2) =0.693/ (K)

Data were statistically evaluated by one-way analysis of variance (ANOVA). All statistical analysis was done using the statistical package for social sciences (SPSS 16.0) program.

3.RESULTS AND DISCUSSION:

3.1.Method validation:

The calibration curve of sulfur showed strong correlation between concentrations and area in the studied range (0–100 ng/ mL) ($r^2 > 0.995$). The LODs and LOQs were sufficiently low; 0.05 **µg** / kg and 0.1 **µg**/ kg, respectively. These limits are, in all cases, below the maximum residue limits (MRLs) established by **(Pesticide EU-MRL Regulation (EC) No. 396, 2005)** at 50 mg/ kg for fruits and leaves. The method had a good repeatability expressed by the relative standard deviation (RSDs) < 14 %. The average recoveries ranged from 96.3%–99.4% in all cases, with RSD lower than 9.5 %.

3.2.Sulfur residues in/on grape fruits and leaves:

The dissipation trends of sulfur residues mg/kg in/on grape fruits and leaves were shown in Table 1. Sulfur dissipated rapidly after application. The concentration of Sulfur in/on grape fruits and leaves after 1 h treatment were 22.4 and 53.73 mg/kg.

Rapid losses of sulfur on these grape fruits and leaves were observed within one day following application. The residues were decreased to 18.66 and 48.66 ppm with a loss of 16.69 and 9.43 % within the first 24 hours after application. The residues of sulfur in grapes fruits were dropped to 10.2, 5.3, 3.3, 0.116 and 0.005 mg/kg with, % dissipation 54.44, 76.33, 85.26, 99.48 and 99.77 % after 3, 5, 7, 12 and 15 days, respectively. Samples taken 21 and 29 days after treatment contained no detectable amount of sulfur in grapes fruit.

Also the data indicate that the persistence of sulfur in grapes leaves were decreased to 33.59, 22.48, 15.30, 12.66, 11.04, 10.22 and 9.96 mg/kg and

% dissipation 37.48,58.16,71.52,76.43,79.54,80.97 and 82.20 % after 3, 5, 7, 12 ,15,21and 29 days, respectively .

These values were less than the maximum residual level MRL 50 mg/kg **(Pesticide EU-MRL Regulation (EC) No. 396, 2005)**. This finding clearly indicated that ,the grape fruits and leaves were could be marketed with apparent safety for human consumption directly after 0 and one day of application .In addition residue half-life values in the grape fruits and leaves were 2.57 and 4.6 days, respectively.

Our findings agree with [5 and 9]. Who reported that, the tomato and squash fruits could be marketed with apparent safety for human consumption directly after application .In addition residue half-life values in tomato and squash fruits were 6.28 and 2.6 days, respectively **(NASR, 2010)**. While **(Cassidy, 1976)** shows that, sulfur exhibits a pronounced reversed-phase effect on styrene-divinylbenzene packings and this selective interaction can be used for the analysis of elemental sulfur by high-speed liquid chromatography. Absorption at 254 nm offers sensitive detection (1-10 ng) and calibration curves are linear up to 10-20 µg. Examples are given for the analysis of sulfur in oil and in aqueous media. Some of the parameters (packing pressure, column diameter and pore size) have been examined for this small (15-37 µm), spherical packing and a recommended packing procedure is given.

Table 1: Dissipation of Sulfur residues (mg kg−1) in/on grape fruits and leaves

Time (days)	Residue level (mean)on fruits	% Dissipation	Residue level (mean)on leaves	% Dissipation
Zero time	22.4	00	53.73	00
One day	18.66	16.69	48.66	9.43
3days	10.2	54.44	33.59	37.48
5days	5.3	76.33	22.48	58.16
7days	3.3	85.26	15.30	71.52
12days	0.116	99.48	12.66	76.43
15days	0.05	99.77	11.04	79.45
21 days	BLD	-	10.22	80.97
29 days	BLD	-	9.56	82.20
MRL	50			
$T_{0.5}$	2.57		4.6	
PHI	0		1	

BLD= blow limited of detection

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تحديد الكبريت المضاف إلى العنب باستخدام تقنية الكروماتوغرافيا السائلة عالية الأداء مع الكشف بواسطة مصفوفة الثنائيات (Diode-Array)

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قسم بحوث متبقيات المبيدات و تلوث البيئة ، المعمل المركزى للمبيدات ، مركز البحوث الزراعية ، الدقى ، الجيزة ، مصر

الملخص العربى:

طُوّرت طريقة مُعتمدة للكروماتوغرافيا السائلة عالية الأداء مع كاشف مصفوفة الصمام الثنائي لتحديد الكبريت في العنب في وقت واحد. قُدّر حدّ الكشف وحدّ التقدير الكمّي للكبريت بـ ٠,٠١ و٠,٠٥ ملغ/كجم على التوالي. تراوحت نسب الاسترداد بين ٩٦,٣% و٩٩,٤%. وُجد أن متوسط الترسب الأولي للكبريت في ثمار العنب وأوراقه بلغ ٢٢,٤ و٥٣,٧٠ ملغ/كجم على التوالي عند معدل تطبيق واحد. أظهرت تجارب التبديد أن نصف عمر الكبريت (T1/2) كان حوالي ٢,٥٧ و٤,٦ يوم في عينات ثمار العنب وأوراقه على التوالي. ووفقًا للحد الأقصى للبقايا (MRL)، كانت فترة ما قبل الحصاد (PHI) للكبريت في ثمار العنب وأوراقه ٠ و٢ يوم بعد المعالجة.